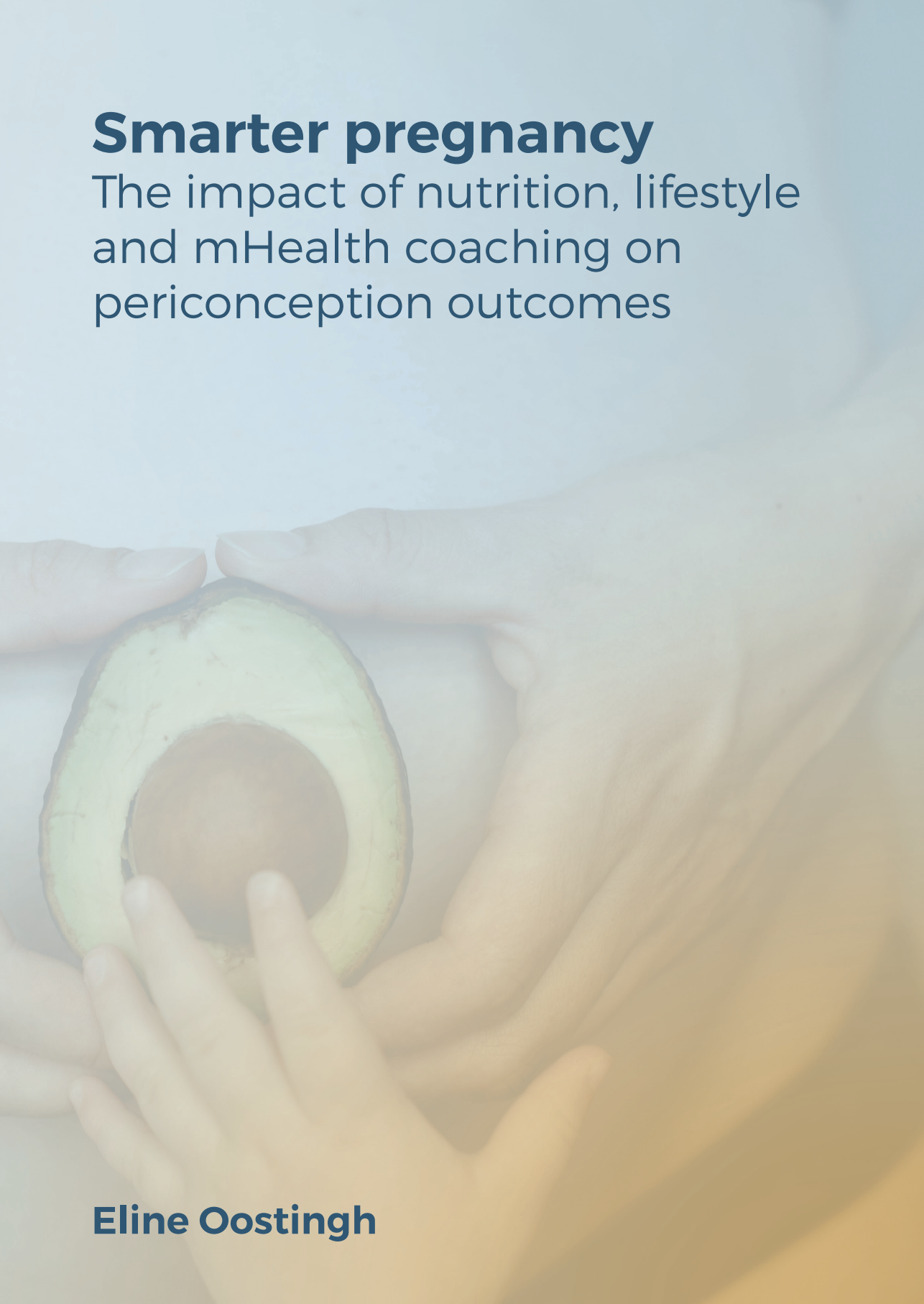
Smarter pregnancy

The impact of nutrition, lifestyle and mHealth coaching on periconception outcomes



Eline Oostingh

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Elsje Cornelia Oostingh

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Smarter pregnancy
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Slimmer zwanger
De invloed van voeding, leefstijl en mHealth coaching op periconceptionele uitkomsten

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Voor pap en mam

Contents

Part III

Addendum

Introduction

Rationale

Worldwide, almost 50 million couples are coping with subfertility (1), a disease of the reproductive system defined by the World Health Organization (WHO) as the failure to achieve a clinical pregnancy after more than 12 months of regular unprotected intercourse (2). To assess whether female or male factors or a combination of both are the underlying cause of the subfertility, a routine fertility work-up has to be performed. Besides medical history taking a physical examination is performed which includes several laboratory tests, ovulation monitoring via ultrasound, tubal patency assessments and a semen analysis. The latter consists of a standardised analysis of the ejaculate volume, sperm concentration, total sperm count (as a product of ejaculate volume and concentration), motility, morphology, and total motile sperm count (as a product of total sperm count and percentage progressive motile sperm).

Depending on the cause, subfertility can be treated using different artificial reproductive technology (ART) modalities to enhance the chance of an ongoing pregnancy. Treatment modalities include ovulation induction to restore normal ovulation in anovulatory patients, artificial insemination or in vitro fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI) to aid the fertilisation of the oocyte by sperm. Besides the underlying medical causes and the aforementioned routine reproductive treatments, increasing attention is paid to poor nutrition and lifestyle behaviours that similarly impact on fertility. Indeed, improvement of these modifiable behaviours significantly increases the chance of an ongoing pregnancy.

The possible impact of nutrition and lifestyle behaviours on reproductive health and health outcomes in later life has been postulated for quite some time. In the late 1890's 'Villa Dijkzigt', on the land of Hoboken, was utilized to educate female citizens of Rotterdam about public health, healthy nutrition and how to achieve a healthy pregnancy. Moreover, the ancient Greek believed that looking at statues or other artworks during pregnancy would lead to the birth of a beautiful child, also, in the eighteenth century inhabitants of Great Britain believed that cravings experienced by a pregnant woman could leave a permanent mark on her offspring (3, 4). Nowadays, knowledge has expanded and the importance of healthy nutrition and lifestyle behaviours is especially designated to a specific critical timespan in life; the

periconception period. This period, defined as the 14 weeks before up to 10 weeks after conception, is known to be very important for human development because it covers the biological processes of gametogenesis, fertilization, embryogenesis and placentation (5).

Gametogenesis is the biological process in which primary oocytes mature into ova and spermatids into spermatozoa. Modifiable behaviours, such as poor nutrition and lifestyle, can lead to derangements of these complicated and complex processes probably through production of reactive oxidative radicals and alterations in DNA synthesis and repair as result. One of the common pathways involved in these molecular biological processes is the one-carbon metabolism. One-carbon units are, amongst others, essential for synthesis en methylation of RNA and DNA, and phospholipid and protein biosynthesis. For a proper regulation of this metabolism, substrates (folate, methionine) and co-factors (cobalamin, vitamin B6 en B2), which are provided by healthy well-balanced nutrition, are crucial. Besides poor nutrition, lifestyle factors such as smoking and excessive coffee and alcohol consumption also lead to derangements in the one-carbon metabolism, as shown by elevated plasma homocysteine levels. Several studies have shown that hyperhomocysteinaemia is associated with impaired oocyte and embryo quality and subsequent reproductive failures (5-7).

Hence, nutrition and lifestyle behaviours do not only affect fertility, but can also derange epigenetic programming affecting the growth and development of the embryo with long-term life course and even transgenerational consequences for health (8-10). The paradigm of the Barker hypothesis, years later followed by the developmental origins of health and disease (DOHaD) theory, implies that a poor intra-uterine environment leads to permanent alteration of the structure, physiology and metabolism of the offspring (11, 12). The Dutch Famine birth cohort study supports this hypothesis by showing an increased prevalence of chronic disease (e.g. glucose intolerance and coronary heart disease) in later life in offspring of mothers who were pregnant during the Dutch famine of 1944-1945 and were thus exposed to starvation (13, 14). This permanent alteration is often reflected by impaired prenatal growth in second half of pregnancy.

Rapid developments in ultrasound equipment and in particularly of the combination of 3D ultrasound and virtual reality as developed by the Erasmus University Medical Centre, have made an enormous contribution to enhance the resolution and visualization of the human embryo, thereby providing new possibilities for research and future early pregnancy care (15). Virtual reality enables visualization and qualification of morphology as well as biometric and volumetric measurements of an embryo in vivo (16, 17). First trimester embryonic size and growth is determined by crown-rump length and embryonic volume. Aberrant growth this early in pregnancy is associated with an increased risk of adverse outcomes, such as congenital malformations, preterm birth, and being born small for gestational age (18-20). All studies presented in this thesis will use the aforementioned techniques to assess these embryonic dimensions.

Summarizing, subfertility is an important global health and societal burden on which modifiable factors such as poor nutrition and lifestyle behaviours have a significant impact. Adhering to more healthy behaviours is of major importance for both women and men as it affects both short and long term outcomes such as gamete quality, embryonic growth, pregnancy outcome and health in later life of the offspring. Therefore, it is of utmost importance that couples contemplating pregnancy are aware of the beneficial effects of healthy nutrition and lifestyle and change their unhealthy behaviours prior to conception, thus in the preconception period. Preconception care (PCC), which has the objective to prevent defects and diseases of mother and child to be by detecting risk factors prior to conception (21), is the opportunity to inform and motivate couples to make such behavioural changes. However, in clinical practice, counselling is limited as several barriers for PCC exist, such as lack of knowledge of health care providers, lack of standardized guidelines, and lack of time and financial resources (22, 23).

A potentially effective and modern alternative to reach couples contemplating pregnancy, are mobile health (mHealth) interventions (24-26). mHealth applications do have the potential to transform health care delivery into an easy access method and offers a way to anonymously control and self-manage information for adopting towards more healthy behaviours (25, 27). In 2011, the Erasmus University Medical Centre Rotterdam launched the mHealth coaching program Smarter Pregnancy as an online platform to provide healthcare workers with a user-friendly and evidence based tool to facilitate

preconception care and to stimulate couples who are contemplating pregnancy to adopt healthy nutrition and lifestyle behaviours (25). After the successful survey on feasibility, usability, and first effectiveness, a randomized controlled trial was performed to study the effectiveness of the mHealth coaching program Smarter Pregnancy (28).

Aims of this thesis

In this thesis we aim to investigate the impact of periconception parental nutrition and lifestyle behaviours on fertility and embryonic growth and to what extent these behaviours can be improved by mHealth intervention.

The key objectives of this thesis are:

1. To investigate the impact of maternal nutrition and lifestyle behaviours on periconception outcomes.
2. To study associations between periconception paternal nutrition and lifestyle behaviours on semen quality and embryonic growth.
3. To assess the (cost) effectiveness of the mHealth coaching platform Smarter Pregnancy.

Methodology

The studies described in this thesis were conducted within the Division of Reproductive Endocrinology and Infertility of the Department of Obstetrics and Gynaecology of the Erasmus University Medical Centre, Rotterdam, the Netherlands.

The Rotterdam Periconception Cohort

From 2009 the Rotterdam Periconception Cohort (Predict study) is an ongoing tertiary hospital-based cohort conducted at the Erasmus University Medical Centre in Rotterdam in which women and their male partners are enrolled during the periconception period and followed up until 12 months after birth (29). Pregnant women of at least 18 years of age receive serial 3D ultrasound scans between 6-12 weeks of gestation. These scans are performed using a 6-12 MHz transvaginal transducer of the Voluson E8 system (General Electrics Medical Systems, Zipf, Australia). Subsequently, measurements of crown-rump length and embryonic volume are performed offline on 3D ultrasound volumes using 4D View software (General Electrics Medical Systems, Zipf, Australia) and the BARCO I-space virtual reality system (Barco N.V., Kortrijk, Belgium).

At study entry, data on maternal and paternal characteristics, medical (obstetric) history and lifestyle are collected through self-administered questionnaires and verified by a researcher. A validated semi-quantitative food frequency questionnaire is used to obtain detailed information on habitual food intake of the previous four weeks. Information on semen quality is not routinely collected as part of the Predict study, but can be retrieved from medical records.

Smarter Pregnancy IVF/ICSI-trial

Smarter Pregnancy (www.slimmerzwangeronderzoek.nl) is an online, mHealth coaching program to improve unhealthy nutrition and lifestyle behaviours of couples contemplating pregnancy. In several fertility clinics throughout the Netherlands (Erasmus University Medical Centre Rotterdam, Reinier de Graaf Gasthuis Delft, Academic Medical Centre Amsterdam, Utrecht University Medical Centre, Groningen University Medical Centre, Leiden University Medical Centre), couples with an IVF or ICSI-indication were invited to participate if they were to start their treatment within three months. At study entry, all participants completed the online baseline screening on nutrition and lifestyle behaviours. Based on inadequate behaviours the six months coaching was generated subsequently. Women and men assigned to the intervention group received tailored coaching consisting of a maximum of three interventions per week, comprising email messages with feedback, tips, recommendations, additional questions addressing behaviour, pregnancy status, body mass index (BMI) or adequacy of the diet, and incentives such as vouchers and seasonal recipes. Besides, after 6, 12, 18 and 24 weeks of coaching participants were asked to complete follow up questionnaires to monitor changes in nutrition and lifestyle behaviour and pregnancy status. Women and men assigned to the control group did not receive tailored coaching after the baseline screening, they only received one seasonal recipe per week and were asked to complete the monitoring questionnaire at time points 12 and 24 weeks without receiving any feedback on these questionnaires.

Outline of this thesis

Part I comprises research on parental periconception nutrition and lifestyle behaviours, with a focus on reproductive outcomes and embryonic growth. In ***Chapter 2*** we present the results of a systematic literature review on the impact of maternal environmental exposures on periconception outcomes. Thereafter, the impact of paternal exposures on reproductive outcomes will be discussed. To start, in ***Chapter 3*** the association between paternal dietary patterns and semen quality is evaluated. This is followed by ***Chapter 4*** in which the association between paternal dietary patterns and embryonic growth is described. Lastly, the influence of paternal use of sympathomimetics in the preconception period on semen parameters is addressed in ***Chapter 5***.

In ***Part II*** we studied the (cost-) effectiveness of the mHealth coaching program Smarter Pregnancy. In ***Chapter 6*** the study design of the Smarter Pregnancy randomized controlled trial is presented. Subsequently, ***Chapter 7*** describes the results of this randomized controlled trial, focussing on the improvement of inadequate nutrition and lifestyle behaviours in couples undergoing IVF/ICSI treatment. This part ends with ***Chapter 8*** covering a description of a cost-effectiveness model of the mHealth coaching program Smarter Pregnancy.

Part III includes the general discussion of the main findings and suggestions for further research (***Chapter 9***). Furthermore, in ***Chapter 10*** a summary of this thesis is provided in English and in Dutch.

The impact of maternal lifestyle factors on periconception outcomes

a systematic review of observational studies

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2

Elsje C. Oostingh
Jennifer Hall
Maria P.H. Koster
Bola Grace
Eric Jauniaux
Régine P.M. Steegers-Theunissen

Abstract

Main risk factors for important reproductive health issues such as subfertility and perinatal mortality largely originate in the periconception period. To evaluate associations between modifiable maternal lifestyle factors and periconception outcomes, we conducted a systematic search for relevant studies published from 1990 to February 2017 on Embase, Medline, PubMed, Web of Science, Cochrane database, PubMed, and Google Scholar. The initial search identified 6166 articles out of which 49 studies were eligible for inclusion.

Fecundity (the capacity to have a live birth) showed significant inverse associations with smoking, alcohol use and poor diet. Studies regarding time to pregnancy showed a decline in fecundability ratios (the monthly conception rate among exposed relative to unexposed couples) with increasing body mass index (BMI). Furthermore, risk of first-trimester miscarriage was found to be increased in smokers, when consuming alcohol and caffeine, and with increasing BMI. Vitamin supplement use showed a decrease in this risk.

This review demonstrates that maternal modifiable lifestyle factors have impact on periconception outcomes. If couples planning a pregnancy are more aware and supported to adopt healthy lifestyles during the periconceptional 'window of opportunity', short-term reproductive health as well as health in later life and even of future generations can be further improved.

Key Message

In this systematic review of observational studies, modifiable maternal lifestyle factors were found to influence several periconception outcomes. This data further support the importance of adopting healthy lifestyles of couples planning a pregnancy to improve reproductive health.

Introduction

Ravelli et al. (30) were one of the first to show increased rates of obesity as a composite determinant of poor lifestyles, in individuals who had been exposed to famine in utero. The link between early-life environment and adult disease was subsequently investigated in women exposed to famine in the Dutch hunger winter during the last winter of the Second World War, showing that offspring exposed to starvation in utero indeed had an increased risk of metabolic and cardiovascular diseases in adulthood (13, 31). In the 1980s, this concept was developed by David Barker, who reported for the first time a negative correlation between low birth weight and the rate of death from ischemic heart disease (32, 33). He also hypothesized that low birth weight in offspring, as a proxy for poor prenatal maternal nutrition, not only increases the risk of coronary heart disease in adulthood, but also of other non-communicable diseases (NCDs), such as obesity and certain cancers (32-34). To explain these findings, it was suggested that, due to plasticity, fetuses can adapt to the environment they expect to enter into once outside the womb. This has been the basis for the hypothesis of the Developmental Origins of Health and Disease (DOHaD) (35).

The DOHaD paradigm focusses mainly on exposures during pregnancy and outcomes at birth and in later life. However, many adverse pregnancy outcomes, such as subfertility, congenital malformations, low birth weight and preterm birth, originate in the periconception period, a critical window which has been neglected in both research and patient care. Therefore, based on molecular biological processes and epigenetics, we have defined the periconception period as a time span of 14 weeks before to up to 10 weeks after conception (5). During this critical period, fertilization, implantation, and development and growth of the embryo and placenta take place (36, 37). This window is therefore pivotal to human reproduction in general and pregnancy outcome in particular. The periconception environment is determined by maternal pre-existing medical conditions and modifiable lifestyles, including smoking, diet and body mass index (BMI) (38). The prevalence of poor lifestyle behaviors in the reproductive population is comparable to the prevalence in the general population (39). There is growing evidence about the impact of lifestyle factors on fertility in women of reproductive age (40, 41). Being obese or overweight before conception is thought to exert a negative influence on female fertility due to dysregulation of the hypothalamic-pituitary-ovarian axis leading

to ovulatory dysfunction (42). Excessive gestational weight gain and obesity during pregnancy are key predictors of childhood obesity and of metabolic complications in adulthood (43). Children of women who are overweight or obese from the beginning of pregnancy are also at increased risk of cognitive deficits, externalizing problems (particularly attention-deficit/hyperactivity disorder), and internalizing psychopathology in childhood and adolescence (44). Besides BMI, smoking is another common lifestyle factor affecting both fecundity (45) and embryonic growth during the first six months of life (46). These data suggest an extension of the window of opportunity for prevention and intervention in to the earliest moments of life.

Before the advent of high-resolution ultrasound, and in particular of three-dimensional ultrasound, in vivo data on embryonic and placental development during the first trimester of pregnancy was limited. These non-invasive technique have now provided large databases on normal and abnormal feto-placental development, thus enabling a better understanding of the pathophysiology of the early embryonic development and its possible impact during pregnancy and after birth (47-49). This has also stimulated periconceptional prospective research on the influence of maternal lifestyle factors on the risk of first trimester abnormal outcomes, mainly miscarriage, congenital malformations and embryonic growth (50-52).

The awareness of the importance of the periconception period is rising, resulting in more published research on this topic. The aim of this review was to provide a systematic and detailed analysis of the literature on maternal lifestyle factors during the periconception period and their impact on fecundity and time to pregnancy, as preconception outcomes, and on miscarriage and embryonic growth as first-trimester pregnancy outcomes.

Materials and Methods

Systematic review information sources and search strategy

The literature review was conducted using the 'Meta-analysis of Observational Studies in Epidemiology (MOOSE)' guidelines (53). Searches were carried out using the electronic databases Embase, Medline, PubMed, Web of Science, Google Scholar and Cochrane databases. The search protocol was designed a priori and registered with the PROSPERO registry (PROSPERO 2016: CRD42016046123). The search strategy

consisted of MeSH terms and keywords for lifestyle exposures of interest, including diet, smoking, alcohol, folic acid / vitamin supplement use, physical activity, and obesity (***Supplemental Table 1***)**.** These were combined using the Boolean operator 'or'.

Systematic review eligibility criteria and used definitions

The periconception outcomes, as defined in the International glossary on infertility and fertility care, 2017 (54), were:

- Fertility: the capacity to establish a clinical pregnancy.
- Fecundity: the capacity to have a live birth.
- Fecundability: The probability of a pregnancy, during a single menstrual cycle in a woman with adequate exposure to sperm and no contraception, culminating in live birth. Frequently measured as the monthly probability.
- Fecundability ratio: the monthly conception rate among exposed relative to unexposed couples.
- Time to pregnancy (TTP): the time taken to establish a pregnancy, measured in months or in numbers of menstrual cycles.
- Miscarriage: spontaneous loss of a clinical pregnancy before 22 completed weeks of gestational age. In this review however, only first-trimester miscarriages (until the 12th week of gestation) were taken into account.
- Embryonic growth: the process by which the embryo forms and develops. In this review only growth, measured by crown-rump length (CRL) was taken into account. For embryo development the Carnegie stages were used.
- Yolk sac: a membranous sac attached to the embryo, formed by cells of the hypoblast adjacent to the embryonic disk. In this review the size of the yolk sac was taken into account.

We found that the terms fertility, fecundity and fecundability were used interchangeably in the literature. We therefore included all terms in the literature search and excluded papers that only provided data on birth outcomes. We did not expect to find literature on congenital malformations and placental size in the first trimester, therefore we did not include those keywords in the literature search. The results of all the periconception outcome searches were combined with 'or'. The results of the separate lifestyle factors and periconception outcome searches were then combined with 'and'.

Inclusion and exclusion criteria

Observational studies of any design that investigated the relationship between maternal lifestyle factors and any of the periconception outcomes of interest were eligible for inclusion in the review. The periconception period was defined as the 14 weeks before and 10 weeks after conception (5). Articles published between 1990 and February 2017 were included and our search was limited to articles published in English. We excluded animal studies and those focused on IVF/ICSI-treatment, male lifestyle factors, semen parameters, congenital anomalies or teratogenicity. Articles that only reported outcomes in the second or third trimester or later life, editorials and review articles were also excluded.

Full text review and data extraction

Title, abstracts and full-text articles were independently assessed for content, data extraction and analysis. References of included studies were also reviewed. ECO reviewed the titles and abstracts and selected papers for full-text review. Full-text review and data extraction was completed by ECO, JH and BG, with all papers reviewed by at least two people. Data were inputted into a template designed specifically for this review. Differences were resolved by discussion between these three authors. Data extracted included the location, year of publication, study design, setting, study population, sample size, exposures of interest, outcome data, exclusion criteria, statistical analysis, potential confounders, results, and conclusion.

Quality of study and risk of bias

The ErasmusAGE quality score for systematic reviews was used to assess the quality of studies included in our review (***Supplemental Table 2***). This tool is based on previously published scoring systems (55, 56) and is composed of five items covering study design, study size, method of measuring exposure and outcome, and analysis. The parameters for these items can be adapted, based on literature and discussion with experts, as relevant for each review. The parameters chosen for our review are shown in ***Supplemental Table 2***. Each item was allocated zero, one or two points giving a total score between zero and ten, with ten representing the highest quality.

Results

Results of search and description of studies

Figure 1 summarizes the process of literature identification and selection of studies. The initial search identified 10,696 records of which 4,530 were duplicates. Of the remaining 6,166 records, a total of 6,012 publications were excluded because they did not fulfil the selection criteria. The full text of 154 papers were read, 105 papers were excluded leaving 49 articles for analysis.

The characteristics of the included studies are shown in ***Table 1***. Thirty-five studies were identified as prospective, and six as retrospective cohort studies, and three and five studies as prospective and retrospective case-control studies, respectively. The search term yolk sac size yielded no results, therefore this parameter is not included in the review.

Fecundity

Nine studies reported associations between maternal lifestyle factors and fecundity (57-65) (***Table 2***). The impact of smoking was evaluated in three studies, all showing poorer fecundability ratios with higher levels of smoking (59-61). The association between alcohol and fecundity was evaluated in three studies (58, 62, 63) and showed lower conception rates with the consumption of alcohol. There was no significant relationship between caffeine consumption and conception rates in the two studies investigating this outcome (63, 65). The association of diet was evaluated in two studies (57, 60). Toledo et al. (57) found that stronger adherence to the Mediterranean dietary pattern was associated with significantly lower odds of consulting a physician because of failure to conceive. The possible negative association of consuming fish from the Baltic sea contaminated with persistent organochlorine compounds was evaluated by Axmon et al. (60). This study found a significantly lower pregnancy success rate ratio in women living in the east coast of Sweden, where higher blood levels of persistent organochlorine compounds have been found, compared to women living in west coast. Folic acid and multivitamin supplement use were both found to be associated with increased fecundity (64).

Time to pregnancy

The association between maternal lifestyle factors and time to pregnancy was evaluated in nineteen studies (66-84) (***Table 3***). Six studies evaluated the impact of smoking on time to pregnancy (69, 70, 73, 75, 77, 81), all showing a prolonged time to pregnancy among smokers.

The possible association of alcohol consumption and time to pregnancy was also reported in six studies (69, 71, 72, 75, 77, 80), but showed inconsistent results. Mutsaerts et al. (69) and Axmon et al. (77) reported that women consuming >7 units of alcohol per week have a significantly longer time to pregnancy compared to women consuming less units per week whereas Juhl et al. (71, 72), reported a slightly shorter time to pregnancy for women consuming alcohol weekly compared to drinking no alcohol.
The association of consumption of caffeine and time to pregnancy was addressed in four studies (74-76, 83). Significant increases in time to pregnancy were found for those women drinking ≥501 mg caffeine per day (76). By contrast, Florack et al. (75) showed a significant decrease when drinking 3-7 cups of caffeine drinks per day compared to drinking <3 cups.

The association of diet and vitamin supplement use was evaluated in four studies; however, none of the results were statistically significant (69, 77, 78, 82). Overall, there was a suggestion of shorter time to pregnancy when using vitamin supplements. By contrast, vitamin D deficiency does not seem to prolong the time to pregnancy.
Six studies reported on the association of BMI and time to pregnancy, showing consistently prolonged time to pregnancy in overweight or obese women (66-70, 79).
The association of physical activity was evaluated in three studies (69, 79, 84). In one study, vigorous physical activity was found to be associated with a prolonged time to pregnancy, in all other studies no association with time to pregnancy was found.

Miscarriage

Fourteen studies evaluated the association between maternal lifestyle factors and first trimester miscarriage (85-98) (***Table 4***). The impact of smoking was evaluated in three studies (85, 94, 97) all showing a statistically significant increase in risk of miscarriage in smokers.

The seven studies reporting on the association between maternal alcohol consumption and miscarriage showed inconsistent results (85-87, 89, 93, 96, 97). The study with the highest quality reported no association between binge drinking in the first 12 weeks of pregnancy and the risk of spontaneous miscarriage (87). This finding is supported by a hospital-based case-control study among Chinese women (85) and by Parazzini et al. (97). In contrast, Windham et al. (86) found a significant association for drinking >3 drinks per week and the risk of spontaneous miscarriage. A similar significant association was found by Kesmodel et al. (89) and Feodor Nilsson et al. (93).

The association between maternal caffeine consumption and miscarriage was evaluated by four studies consistently reporting inverse associations (90, 93, 94, 97), though not all were statistically significant.

The impact of diet was evaluated in one study (85). The authors reported on the association of eating fresh fruit/vegetables on a daily basis compared with not eating fresh fruit/vegetables daily and the risk of miscarriage and they found no significant reduction in risk.

Four studies examined the association between folic acid and / or vitamin supplement use and miscarriage (85, 88, 92, 95). Ronnenberg et al. (88) showed a positive trend for an increase in the relative odds of spontaneous miscarriage as plasma folate concentration decreased, which was weakened after adjusting for confounders. A borderline significant increase in risk of miscarriage was seen for Vitamin B6 status (p for trend 0.06) but this also diminished after adjustment. However, comparing Vitamin B6 status between women whose pregnancies ended in a clinically recognized spontaneous miscarriage and in those with live births, showed a significantly ($p = 0.04$) lower mean pre-pregnancy plasma Vitamin B6 concentration in women with miscarriage. This finding is supported by a case-control study among Chinese women showing a significant reduction in risk for miscarriage among women using multivitamin supplements compared to those without using supplements (85).

The association between BMI, physical activity and miscarriage was evaluated in five studies (85, 91, 93, 97, 98). Higher BMI was shown to increase the risk of miscarriage, whereas moderate physical activity decreased the risk of miscarriage.

Embryonic growth

The association between maternal lifestyle factors and embryonic growth was reported in seven studies (50, 99-104) (***Table 5***). Van Uitert et al. (50) showed that periconception smoking and periconception alcohol use were independently associated with reduced embryonic growth trajectories, measured by CRL. No associations were observed with BMI and timing of folic acid supplement use. Bakker et al. (101) evaluated the impact of caffeine; intake of >6 cups per day was associated with a decline in CRL.

Evaluation of maternal red blood cell (RBC) folate levels in the first-trimester as a measure of nutrition and supplement use showed an optimum use curve, in which both lower and very high levels are associated with reduced embryonic growth (102). Another study showed that smoking in combination with lack of use of folic acid supplements was associated with reduced embryonic size (104). This association between smoking and embryonic size was not found by Prabhu et al. (99). Increasing adherence to an energy-rich dietary pattern is significantly associated with an increased CRL, as reported by Bouwland-Both et al. (100).

Association between embryonic morphological development according to the Carnegie stages and maternal biomarkers of the one carbon metabolism was evaluated in the study by Parisi et al. (103). Low vitamin B12 concentrations (-2SD, corresponding to 73.4 pmol/l) were associated with a 1.4-day delay in morphological development compared with high concentrations (+2SD, corresponding to 563.1 pmol/l) and high total homocysteine concentrations (+2SD, corresponding to 10.4 µmol/l) were associated with a 1.6-day delay in morphological development compared with low concentrations (-2SD, corresponding to 3.0 µmol/l).

Discussion

The results of our systematic review highlight the impact of maternal modifiable lifestyle factors including smoking, alcohol, caffeine, BMI, physical activity, diet and vitamin supplement use on fecundity and first trimester pregnancy outcomes.

Smoking

Cigarette smoke contains about 4,000 compounds belonging to a variety of chemical

classes known to be toxic, including polycyclic aromatic hydrocarbons (PCH), nitrosamines, heavy metals, alkaloids, aromatic amines and so forth (105). The exact mechanism remains unclear but there is strong evidence that these constituents may affect the follicular microenvironment and alter hormone levels in the luteal phase (106). These alterations in hormone levels shorten the luteal phase, which results in a shorter time period of being able to become pregnant. Besides, decreased ovarian function and reduced ovarian reserve may also be possible consequences of smoking, as shown by lower Anti-Müllerian hormone (AMH) levels in smokers compared to non-smokers (107). Studies included in this review confirm these hypotheses by showing statistically significant negative associations of smoking especially with fecundity parameters (59-61), although a significantly prolonged time to pregnancy was found in only two out of six studies included in our review (73, 81).

Different compounds of cigarette smoke also impair endometrial maturation, implantation and early placentation (105). Nicotine is suspected to have an adverse effect on the decidualization process and cadmium, for example, is known to impair endometrial maturation. Moreover, several studies have indicated the negative influence of benzo(a)pyrene on angiogenesis by inhibiting endothelial cell proliferation (105). These mechanisms could explain the significant increase in the risk of first trimester miscarriage found in two large studies (85, 94). These associations are dependent on the number of cigarettes smoked per day (85).

Alcohol

Although the evidence of associations between alcohol and reproductive performances are inconclusive, antenatal alcohol consumption is a known teratogen and several studies have reported an association with higher rates of early pregnancy failure and decreased fecundity (106, 108) as supported by two studies included in our review (62, 63). One of the biological explanations for these periconception complications is that hormonal fluctuations, including alcohol-induced increase of aromatization of testosterone leading to increase in estrogen levels, reduces follicle stimulating hormone and suppresses both folliculogenesis and ovulation. Furthermore, alcohol may have a direct association on the maturation of the ovum, ovulation, blastocyst development and implantation (109, 110). As a result of these maturations, time to pregnancy may

be prolonged in women who consume alcohol. In two studies included in this review, time to pregnancy was found to be increased in women who consume alcohol (69, 75). In contrast, two other studies showed a significantly shorter time to pregnancy (71, 72). This contradiction may be due to differences in the populations studied, residual confounding, or the type of alcohol consumed. For example, Juhl et al. (71) found a shorter time to pregnancy among wine drinkers than non-wine drinkers.

Alcohol readily crosses the placenta, which can result in irreversible damage to the placenta and organs of the developing embryo (111). Besides adverse pregnancy outcomes such as stillbirth, preterm birth, intrauterine growth restriction and Fetal Alcohol Syndrome (FAS) Disorders, the risk of miscarriage in the first trimester is also increased. Three out of five reviewed studies indeed showed a significantly increased risk of miscarriage with higher levels of alcohol consumption (86, 89, 93). One other study showed a significant association between a reduced embryonic growth and exposure to alcohol (50). While many studies have demonstrated an association between alcohol and perinatal outcomes, the exact dose-response relationship and the differential effects of different types of alcohol, remain unknown and urgently require further research because of the large number of social alcohol consumers in the reproductive population.

Caffeine

It has been hypothesized that caffeine could affect female reproduction by increasing estrogen production and thereby affecting ovulation (112) and corpus luteal function (106), resulting in an increase of the time to pregnancy (113). Caffeine is known to pass the placental barrier and may lead to vasoconstriction of the uteroplacental circulation affecting embryonic and placental growth and development (114). Furthermore, during pregnancy the rate of caffeine metabolism decreases and the half-life doubles, leading to higher exposure of the embryo (114).

A possible explanation for the heterogeneous results of the time to pregnancy in studies included in the present review (74-76) may be that studies did not always control for residual confounding such as smoking, which, is known to be highly correlated with caffeine consumption. Moreover, the rate at which caffeine is cleared from the body,

which varies between individuals and is affected by environmental factors such as smoking and diet (115), may influence the biologic dose and exposure interval. Although these hypothesized mechanisms may explain the association found between caffeine consumption and the increased risk of miscarriage (90, 93, 94), reverse causation must be taken into account. It is known that pregnancy symptoms such as nausea and vomiting, which may cause women to consume less caffeine, are more common in healthy pregnancies that result in live births than when a pregnancy ends in a miscarriage (74-76, 115).

Diet

Diet is known to affect female fecundity (106, 113). In women of reproductive age, the adherence to the Mediterranean diet (characterized by high consumption of vegetables, fish, fruits, poultry, low-fat dairy products, and olive oil (57)) reduces the risk of weight gain and insulin resistance (116) and increases pregnancy rates by 40% in couples undergoing IVF/ICSI (117). Olive oil is an important source of linoleic acid, which is known to improve the reproductive process (117). The energy-rich dietary pattern described by Bouwland-Both et al. (100) is significantly associated with embryonic growth, as measured by CRL. Its high methionine content could explain this association, as this is an essential substrate for the one-carbon pathway. Folate, which is a substrate, and other vitamins, such as B6 and B12 which are co-factors for this pathway, could also play a role in biological processes implicated in growth and programming, especially in the periconception period (5). Furthermore, these vitamins are also associated with increased progesterone levels in luteal phase, improved menstrual cycle regularity and normalization of cycle length, which have all been associated with fecundity (64). These findings could explain the positive association of concentration of vitamin B12 on embryonic development (103) and on fecundity (64).

The expected positive association of multivitamin supplement use and a reduced time to pregnancy was not seen in two studies (69, 77). A possible explanation is the low response rate in one study (77) and the fact that the other study was designed for detection of risk factors for child obesity instead of fertility measures (69). Lower miscarriage rates were found with folic acid and/or multivitamin supplement use in all four studies included in this review (85, 88, 92, 95). Vitamin D is also an important contributor to explain some of

the underlying mechanism, as it regulates the synthesis of several hormones including estradiol, progesterone, and human chorionic gonadotrophin by the villous tissue. These hormones are all essential in maintaining the regulation of utero-placental blood flow, the simulation of neovascularization, and maternal immunotolerance to the embryonic allograft (118).

BMI and physical activity

The detrimental effect of being overweight or obese on the time to pregnancy was observed in five out of six studies included in this review (66-70, 79). This is in agreement with a dysregulation of the hypothalamic-pituitary-ovarian axis resulting in abnormalities in secretion of gonadotropin-releasing hormone, luteinizing hormone, and follicle-stimulating hormone leading to anovulation or decreased oocyte quality and decreased endometrial receptivity in obese women (112, 119). Associated hyperinsulinemia is also known to disturb the hypothalamic pituitary gonadal axis. The increased levels of insulin and leptin lead to insulin and leptin resistance which, in the end impairs ovarian function and fertility success rate (117). Besides the detrimental effects on fecundity, obesity is also known to increase the risk of miscarriage. It is thought that insulin resistance may be involved in several mechanisms such as diminished endometrial production of adhesion factors and a lower serum level of immunosuppressive proteins (120). In this review, we found heterogeneous results for miscarriage in the four included studies (85, 91, 97, 98). This can partly be explained by the fact that it is not always clear whether pre-pregnancy or present BMI was used. Furthermore, only one paper obtained direct measurements of weight and height instead of obtaining this information through self-reported questionnaires (98).

A healthy amount of physical activity can be beneficial by leading to relaxation and reducing stress. Vigorous physical activity however, is known to be potentially harmful by exceeding the energy demand over dietary energy intake, thereby resulting in a negative energy balance which results in hypothalamic dysfunction eventually leading to menstrual abnormalities (113). Subsequently, a prolonged time to pregnancy may occur. In this review we found inconclusive associations in studies reporting the association of physical exercise and time to pregnancy (69, 79, 84). Increasing levels of physical activity is known to be associated with an increased risk of miscarriage

(121). The association between physical activity and risk of miscarriage was reported by two studies in this review. One study reported a decreased risk of miscarriage when performing regular exercise (85), whereas the other (93) showed a significant increase in the risk of miscarriage with ascending amounts of exercise in minutes per week. This may be due to the fact that the assessment of exercise and the types and intensity differed between the included studies. Furthermore, not every study has data on factors that may affect the level of exercise, for example, nausea in first trimester.

Strengths and limitations

The present work is the first to systematically review the currently available evidence on the impact of maternal lifestyle factors on periconception outcomes. Although paternal lifestyle factors are known to influence semen quality and quantity and thereby play an important role in the aetiology of periconception outcomes (122, 123), literature on this matter is still scarce. Therefore, we chose to only include literature assessing maternal lifestyle factors.

Previous reviews have focused mainly on outcomes in the second or third trimester, birth outcomes or outcomes in childhood or adult life, thereby ignoring the importance of fecundity, miscarriages and adverse embryonic and placental growth in first trimester. In most of the human studies, data were obtained at birth or after the end of the first trimester of pregnancy, thereby missing the periconception period where most poor perinatal outcomes originate (36, 37). Other strengths of our study are that 35 out of 49 studies included in our review were large, with more than 1000 participants, increasing the power of the studies. Most studies focusing on the impact of periconceptional maternal lifestyle factors have only been performed in the subfertile population (116, 124, 125), whereas in this review studies in the IVF/ICSI-population were excluded making the results more applicable for the general population. Finally, most of the included studies were prospective studies, which reduced the chances of selection bias, recall bias and reverse causation. Nonetheless, prospective studies may be affected by selection bias because they are usually limited to couples planning a pregnancy and thus excluding the large group of couples with an unplanned pregnancy. The chance of inclusion bias, however, was reduced by including studies of countries from all around the world. The retrospective studies may be at higher risk of selection bias because

most of these studies were limited to women who became pregnant, thus excluding less fertile or sterile women. Moreover, It is also known that highly educated people are more often wiling to complete questionnaires (126), giving rise to selection bias.

Despite our extensive literature search, the amount of evidence and its quality was relatively low. From the current literature, no definite conclusions on causal relations can be drawn. There is lack of uniformity in the application of terminology in this field with terms such as fertility, fecundity, fecundability often being used interchangeably and with variations in the definition of time to pregnancy. Observational studies on the impact of alcohol usage, caffeine and smoking are often based on self-reported information giving rise to recall and social desirability bias and are not always supported by biological data, such as cotinine levels for the cigarette exposure. There was also a possible bias of under-reporting negative issues such as smoking and alcohol use in couples trying to conceive, which should be taken into account. Finally, there was inconsistency in how exposures and outcomes were reported. For example, alcohol use was variously coded as grams of alcohol per day, drinks per week, units per week, number of days per week alcohol was consumed or frequency of binge drinking. The same is true for caffeine and smoking. Misclassification of gestational age can occur when using the first day of the last menstrual period due to variation in cycle length. Even when studies only included women with regular cycles of approximately 28 days, misclassification might still be an issue of concern since the postconceptional age is dependent on the timing of ovulation and implementation. Furthermore, miscarriage was often not divided into first- or second-trimester, instead, the whole period until a gestational age of 20 weeks is included. Within this context, we were unable to perform a meta-analysis.

Conclusion

This review shows that several modifiable maternal lifestyle factors are associated with fecundity and other periconception outcomes such as miscarriage, time to pregnancy and embryonic growth. Several studies have indicated that poor lifestyle factors are very common among women of childbearing age and thus remain of major concern (127). The prevalence of smoking by women in reproductive age for example, is the same as for society in general (128), even though it is well known that exposure in utero impairs pregnancy outcome and health in childhood and later life (129). The same applies to

the use of alcohol. Several studies have indicated that, despite public health efforts to increase awareness of the risks associated with drinking during pregnancy, worldwide approximately 10% of pregnancies are alcohol-exposed, and in the European region this is up to 25% (111). This review makes clear that future research is needed to understand the associations between maternal lifestyle factors and periconception outcomes, and should in particular focus on unifying measurements of lifestyle factors and outcomes, thereby enabling researchers to collect data for a robust meta-analysis to calculate risk ratios. Furthermore, causal pathways should be investigated in more detail. Moreover, the data collected in this review suggest that the target window for the investigation of the DOHaD paradigm should be expanded to include the periconception period and support the concept of preconception care accessible to every woman and couple planning a pregnancy.

Overall, the data in the current review indicate that there is urgent need to implement more effective periconception preventative and surveillance strategies. We hope that our data will stimulate a general interest in developing and funding well-designed prospective periconception intervention studies, rather than observational studies, and contribute to a more general awareness in couples planning a pregnancy and the health care professionals supporting them to adopt healthy lifestyles during this critical window of opportunity. They should also be made aware that these adaptations would also reduce subfertility, perinatal mortality and morbidity and subsequent diseases in later life and next generations.

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Authors' roles

RST and EJ conceived and designed the study. EO performed an initial screening on title and abstract of all articles to exclude citations deemed irrelevant. EO, JH and BG independently evaluated all articles and abstracted data. EO, JH, MK, EJ, RST drafted the first version of the manuscript. All authors contributed to the critical revision of the manuscript and approved the final version.

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Conflict of interest

BG is an employee of 'SPD GmBH'. None of the other authors have any conflict of interest related to the discussed topic.

Table 1. Main characteristics of 49 included studies

Study	Year	Country	Study population	Study design	Sample size	Exposure(s)	Outcome(s)	Quality score*
Anderssen et al.	2015	Denmark	Odense child cohort, pregnant women january 2010 - december 2012	Prospective cohort study	1683	Vitamin use	Miscarriage	5
Arakawa et al.	2006	Japan	Women delivering from january 2002 - march 2004 in two Japanese hospitals	Prospective cohort study	180	Diet	TTP	4
Axmon et al.	2000	Sweden	Fishermen's wives from Swedisch east and west coast, born from 1945.	Prospective cohort study	1335	Smoking, Diet	Fertility, TTP	5
Axmon et al.	2006	Sweden	Random sample of women from the general Swedish-population, born from 1960 onwards.	Prospective cohort study	1557	Smoking, alcohol, vitamin use, drug use	TTP	5
Bakker et al.	2010	The Netherlands	The Generation R study; Dutch women who were resident in the study area and who delivered between April 2002 and January 2006	Prospective cohort study	1310	Caffeine	Embryonic growth	6
Bolumar et al.	1997	Spain	Random sample of women 25-44 yrs, five European countries (Denmark, Germany, Italy, Poland and Spain).	Prospective cohort study	3092	Caffeine	TTP	5
Bouw-land-Both et al.	2013	The Netherlands	he Generation R study; Dutch women who were resident in the study area and who delivered between April 2002 and January 2006	Prospective cohort study	847	Diet	Embryonic growth	5
Caan et al.	1998	USA	Volunteer members of the Kaiser Permanente Medical Pro-gram who were trying to conceive (for max 3 months before entering the study).	Prospective cohort study	187	Caffeine	Fecundity	4
Cnattingius et al.	2000	Sweden	Between 1996-1998, Uppsala Sweden, women with spontaneous abortion who presented at the department at 6-12 wks and had a positive pregnancy test	Prospective cohort study	1448	Smoking, caffeine	Miscarriage	6
Cueto et al.	2015	Denmark	The Danish pregnancy planning study (Snart Gravid)	Prospective cohort study	3895	Folic acid, vitamin use	Fecundity	5
Feodor Nilsson et al.	2014	Denmark	Danish national birth cohort. All pregnancies with info on risk factors for miscarriage.	Prospective cohort study	88373	Alcohol, caffeine, physical activity	Miscarriage	6

Florack et al.	1994	The Netherlands	Between june 1987- jan 1989, female workers 18-39 yr, working in non-medical functions at Dutch Hospitals, planning pregnancy	Prospective cohort study	1683	Smoking, alcohol, caffeine	TTP	5
Gaskins et al.	2014	USA	Female nurses 24-44 yr in the Nurses' Health Study II. With no history of pregnancy loss in 1991 and reported at least one pregnancy during 1992-2009	Prospective cohort study	180	Folic acid	Miscarriage	6
Gaskins et al.	2016	USA	Female nurses 24-44 yr in the Nurses' Health Study II. With no history of pregnancy loss in 1991 and reported at least one pregnancy during 1992-2009	Prospective cohort study	1335	Alcohol	Miscarriage	5
Hahn et al.	2015	Denmark	Snart-Gravid study; Danish women 18-40 yr, resident of Denmark, stable relation with male partner, not using fertility treatment, trying to become pregnant.	Prospective cohort study	1557	Caffeine	Miscarriage	6
Hahn et al.	2014	Denmark	Snart-Gravid study; Danish women 18-40 yr, resident of Denmark, stable relation with male partner, not using fertility treatment, trying to become pregnant.	Prospective cohort study	1310	BMI	Miscarriage	6
Hakim et al.	1998	USA	women reproductive age, no contraceptive use, not sterillized.	Prospective cohort study	3092	Alcohol, Caffeine	Fecundity	5
Hatch et al.	2012	Denmark	Danish, 18-40 yrs, male partner, trying to conceive <12 months	Prospective cohort study	847	Caffeine	TTP	5
Hull et al.	2000	United Kingdom	Couples residence in the defined geografic area administered by the Avon Health Authority and if the expected date of birth was between April 1991 - December 1992	Prospective cohort study	187	Smoking	TTP	6
Jensen et al.	1998	Denmark	Danish couples, 20-35 yr, no childeren, trying to conceive for the first time	Prospective cohort study	1448	Alcohol	Fecundity	4
Juhl et al.	2003	Denmark	Pregnant women within the first 24 weeks of pregnancy recruited to the Danish National Birth Cohort in 1997-2000.	Prospective cohort study	3895	Alcohol	TTP	5
Juhl et al.	2001	Denmark	Pregnant women within the first 24 weeks of pregnancy recruited to the Danish National Birth Cohort in 1997-2000.	Prospective cohort study	88373	Alcohol	TTP	5
Kesmodel et al.	2002	Denmark	women attending routine antenatal care at Aarhus University Hospital Denmark fromm 1989-1996	Prospective cohort study	88373	Alcohol	Miscarriage	5

Laurent et al.	1992	USA	20- 54 year old women who were randomly selected to serve as the control group of the Cancer and Steroid Hormone Study coordinated by the Reproductive Health Division of the Center for Chronic Disease Prevention and Health Promotion, Centers for Disease rol, USA	Prospective cohort study	2714	Smoking	Fertility	5
Law et al.	2007	USA	regnant women enrolled in the Collaborative Perinatal Project at 12 study centres across the United States	Prospective cohort study	7327	Smoking, BMI	TTP	5
Lopez-del Burgo et al.	2015	Spain	university graduates from Spain	Prospective case-control study	1372	Alcohol	Fertility	7
McKinnon et al.	2016	USA en Canada	women 21-45 yrs, not using CC, no fertility treatment, stable relation man, planning a pregnancy, not pregnant. PRESTO.	Prospective cohort study	1274	BMI. PA	TTP	6
Mikkelsen et al.	2016	Denmark	women 18-40 yrs, stable relationship male, trying to conceive, no fertility treatment. Snart Gravid.	Prospective cohort study	4210	Alcohol	TTP	6
Mook-Kanamori et al.	2010	The Netherlands	Generation R study, mothers enrolled 2001-20015	Prospective cohort study	1631	Smoking, alcohol, folic acid, BMI	Embryonic growth	8
Mutsaerts et al.	2011	The Netherlands	Pregnant women in Drenthe with the expected date of delivery between April 2006 and April 2007	Prospective cohort study	1924	Smoking, alcohol, vitamin use, BMI, physical activity	TTP	5
Parazzini et al.	1991	Italy	Jan 1987-1988, cases: women ≥2 unexplained miscarriages in frist 3 months of gestation, without full-term pregnancies. Controls: women admitted for normal delivery.	Retrospective case-control study	270	Smoking, alcohol, caffeine, BMI	Miscarriage	5
Parisi et al.	2017	The Netherlands	Predict study. 2010-2014 women with singleton pregnancies.	Prospective cohort study	234	Vitamin use	Embryonic growth	5
Prabhu et al.	2010	United Kingdom	Mothers attending a first trimester dating ultrasound scan	Prospective cohort study	903	Smoking	Embryonic growth	7

Radin et al.	2014	Denmark	female pregnancy planners aged 18–40 years	Prospective cohort study	3298	Smoking	Fecundity	3
Ramlau-Hansen et al.	2007	Denmark	Couples from Danish National Birth with pregnancy(ies) between 1996 -2002	Retrospective case-control study	47835	BMI	TTP	4
Ronnenberg et al.	2002	China	Female textile workers in Anqing, China	Prospective case-control study	458	Folic acid, vitamin use	Miscarriage	5
Sapra et al.	2016	USA	LIFE study 2005-2009. Couples discontinuing CC for becoming pregnant or were off CC for max 2 months. 18-40 yrs, cycle length 21-42 days, not received injectable CC in the past year.	Prospective cohort study	501	Smoking	TTP	6
Somagliana et al.	2016	Italy	regnant women undergoing first trimester screening for aneuploides. Cases: seeking pregnancy 12-24 months. Controls: age-matched conceiving in less than 1 yr	Prospective case-control study	146	Diet	TTP	5
Strandberg-Larsen et al.	2008	Denmark	Danish national birth cohort, women enrolled between 1996 and 2002, interview done mid-pregnancy	Prospective cohort study	89201	Alcohol	Miscarriage	7
Toledo et al.	2011	Spain	Nested case control study selected from a prospective cohort of university graduates.	Retrospective case-control study	2154	Diet	Fertility	5
van Uitert et al.	2013	The Netherlands	Rotterdam Predict study, an ongoing prospective periconception cohort study that is part of the preconception and antenatal care at the outpatient clinics of the Erasmus MC, University Medical Centre Rotterdam. All women who were at least 18 years old with ongoing intrauterine singleton pregnancies of 6–8 weeks of gestation were eligible for participation and recruited in 2009 and 2010. Spontaneously conceived, plus IUI	Prospective cohort study	87	Smoking, alcohol, folic acid, BMI	Embryonic growth	6
van Uitert et al.	2014	The Netherlands	singleton pregnancies recruited in 2009-2010. Predict Study. 77 patients, 440 ultrasounds	Prospective cohort study	440	Folic acid	Embryonic growth	5
Wesselink et al.	2016	USA en Canada	women 21-45 yrs, not using CC, no fertility treatment, stable relation man, planning a pregnancy, not pregnant. PRESTO.	Prospective cohort study	1318	Caffeine	TTP	6

Windham et al.	1997	USA	Women were recruited during 1990-1991 from a large pre-paid health plan (Kaiser Permanente Medical Care Program) in three geographical areas in California, they were informed of the study when they called to make their first antenatal appointment.	Prospective cohort study	5307	Alcohol	Miscarriage	5
Wise et al.	2010	Denmark	Women were part of the the "Snart Gravid" study, an internet-based prospective cohort study of women planning a pregnancy in Denmark. Recruitment began in June 2007. Eligible women were aged 18–40, residents of Denmark, in a stable relationship with a male partner, and not receiving any type of fertility treatment.	Prospective cohort study	1410	BMI	TTP	5
Wise et al.	2012	Denmark	Women were part of the the "Snart Gravid" study, an internet-based prospective cohort study of women planning a pregnancy in Denmark. Recruitment began in June 2007. Eligible women were aged 18–40, residents of Denmark, in a stable relationship with a male partner, and not receiving any type of fertility treatment.	Prospective cohort study	3027	Physical activity	TTP	7
Wise et al.	2013	USA	Women were part of the Black Women's Health Survey, a prospective cohort study of 59 000 African-American women aged 21 to 69 at entry in 1995. This analysis is of the 2011 follow up, where 16462 responded	Prospective cohort study	2022	BMI	TTP	5
Xu et al.	2014	China	Cases - hospitalised in one of 3 hospitals in Zhengzhou City for an early miscarriage (<13wks) from Oct 2009-Dec 2012. 620 cases randomly selected from 3,277, 1,240 agematched controls, post 13 wks, randomly selected from the same period from 21,491 outpatients attending routine prenatal care.	Retrospective case-control study	1860	Smoking, alcohol, diet, vitamin use, BMI, physical activity	Miscarriage	6
Zhou et al.	2016	China	2013-2014 in Anhui China. 18-40 yrs, residents of Anhui, married, not using fertility treatment, trying to become pregnant during the next six months.	Prospective cohort study	2940	BMI	Miscarriage	5

Table 2. Description and summary of data for 9 studies that investigated associations between lifestyle factors and fecundity.

Author	Study design	Sample size	Exposure	Outcome description	Outcome definition	OR (95% CI)
Axmon et al, 2000	Retrospective cohort study	1335	Diet Smoking	consuming contaminated fish from Baltic sea smoking ≥10 cigarettes / day	Success rate ratio (SuRR)	0.86 (0.75 ; 0.99) 0.68 (0.51 ; 0.91)
Caan et al, 1998	Prospective cohort study	187	Caffeine	Intake of caffeine >106.8 mg / day	Relative Odds of becoming pregnant	1.09 (0.63 ; 1.89)
Cueto et al, 2016	Prospective cohort study	3895	Folic acid Vitamin use	use of folic acid supplement in general use of folic acid exclusively use of multivitamin supplements exclusively	Fecundability ratio; the monthly conception rate among exposed relative to unexposed	1.15 (1.06 ; 1.25) 1.15 (1.00 ; 1.31) 1.20 (1.08 ; 1.32)
Hakim et al, 1998	Prospective cohort study	98	Alcohol Caffeine	consuming < 12 grams of alcohol / week consuming 13-90 grams of alcohol / week Intake of caffeine ≥301 mg / day	Relative Odds of conception	0.43 (0.25 ; 0.76) 0.40 (0.21 ; 0.77) 0.83 (0.34 ; 2.01)
Jensen et al, 1998	Prospective cohort study	423	Alcohol	consuming 1-5 units of alcohol / week consuming 6-10 units of alcohol / week consuming 11-15 units of alcohol / week	Odds of conception	0.61 (0.40 ; 0.93) 0.55 (0.36 ; 0.85) 0.34 (0.22 ; 0.52)
Laurent et al, 1992	Prospective cohort study	2714	Smoking	smoking ≥20 cigarettes / day	Odds of primary infertility	1.36 (1.14 ; 1.61)
Lopez-del Burgo et al, 2015	Prospective case-control study	8749	Alcohol	consumption of alcohol ≥ 5 times / week	Odds ratio for presenting with difficulty getting pregnant	1.04 (0.72 ; 1.51)
Radin et al, 2014	Prospective cohort study	3298	Smoking	current regular smoker smoking for ≥10 years	Fecundability ratio; the monthly conception rate among exposed relative to unexposed	0.89 (0.77 ; 1.03) 0.85 (0.72 ; 1.00)
Toledo et al, 2011	Retrospective case-control study	2154	Diet	high adherence to Mediterrean dietary pattern	Odds ratio for presenting with difficulty getting pregnant	0.56 (0.35 ; 0.90)

Table 3. Description and summary of data for 19 studies that investigated associations between lifestyle factors and time to pregnancy.

Author	Study design	Sample size	Exposure	Outcome description	Outcome definition	OR (95% CI)	Other
Arakawa et al. 2006	Prospective cohort study	180	Diet	Geometric means of mercury			2.01 µg/g vs 1.97 µg/g, p-value NS
Axmon et al. 2006	Retrospective cohort study	1557	Alcohol Smoking Vitamin use	Consumption of alcohol smoking cigarettes daily use of vitamin supplements	Fecundability ratio; the monthly conception rate among exposed relative to unexposed	0.83 (0.72 ; 0.95) 0.93 (0.79 ; 1.08) 1.04 (0.89 ; 1.22)	
Bolúmar et al. 1997	Retrospective cohort study	3092	Caffeine	none vs ≥5 cups / day none ≥501 mg /day	Waiting time to first pregnancy (ref category: 6,5 months)		8.2 months, p 0.003 8.9 months, p 0.001
Florack et al. 1994	Prospective cohort study	259	Alcohol Caffeine Smoking	>10 units of alcohol / week 3-7 cups of caffeine drinks / day vs < 3 cups >10 cigarettes / day	Relative Odds of conception	1.2 (0.7 ; 2.3) 1.8 (1.1 ; 3.1) 0.8 (0.5 ; 1.3)	
Hatch et al. 2012	Prospective cohort study	3628	Caffeine	≥300 mg caffeine / day	Fecundability ratio; the monthly conception rate among exposed relative to unexposed	1.04 (0.90 ; 1.21)	
Hull et al. 2000	Prospective cohort study	12106	Smoking	15-19 cigarettes daily conceive within 6 months 15-19 cigarettes daily conceive within 12 month	Odds ratio of taking ≥12 months to conceive	1.47 (1.15 ; 1.87) 1.99 (1.48 ; 2.69)	
Juhl et al. 2001	Retrospective cohort study	29844	Alcohol	7.5-14 units of alcohol / week, conceive after 5 months 7.5-14 units of alcohol / week, conceive after 12 months	Odds ratio for an increasing waiting time to pregnancy	0.84 (0.76 ; 0.93) 0.86 (0.76 ; 0.98)	
Juhl et al. 2003	Retrospective cohort study	29844	Alcohol	>7 units of wine / week	Odds ratio for an increasing waiting time to pregnancy	0.87 (0.78 ; 0.99)	
Law et al. 2007	Prospective cohort study	7327	BMI Smoking	BMI ≥30.0 kg/m2 Among smokers, BMI ≤18.5 kg/m2 BMI 25.0-29.9 kg/m2 BMI ≥30.0 kg/m2	Fecundability ratio; the monthly conception rate among exposed relative to unexposed	0.72 (0.63 ; 0.83) 0.89 (0.78 ; 1.01) 0.97 (0.85 ; 1.11) 0.83 (0.68 ; 1.02)	

McKinnon et al. 2016	Prospective cohort study	1274	BMI Ph. activity	BMI 40-44 kg/m2 BMI ≥45 kg/m2 ≥5 hrs / week vigorous activity	Fecundability ratio; the monthly conception rate among exposed relative to unexposed	0.61 (0.42 ; 0.88) 0.42 (0.23 ; 0.76) 1.11 (0.96 ; 1.28)
Mikkelsen et al. 2016	Prospective cohort study	4210	Alcohol	≥14 units of alcohol / week	Fecundability ratio; the monthly conception rate among exposed relative to unexposed	0.82 (0.60 ; 1.12)
Mutsaerts et al. 2011	Retrospective cohort study	1924	Alcohol BMI Ph. activity Smoking Vitamin use	>7 units of alcohol / week BMI ≥30 kg/m2 ≥4 times / week ≥10 cigarettes / day use of vitamin supplements	Ratio of the 'hazard' of becoming pregnant	0.71 (0.53 ; 0.96) 0.87 (0.76 ; 1.01) 1.04 (0.92 ; 1.18) 0.96 (0.84 ; 1.10) 0.59 (0.86 ; 1.05)
Ramlau-Hansen et al. 2007	Retrospective case-control study	47835	BMI	BMI 25.0-29.9 kg/m2 BMI ≥30 kg/m2	Odds ratio of taking >12 months to conceive	1.27 (1.18 ; 1.36) 1.78 (1.63 ; 1.95)
Sapra et al. 2016	Prospective cohort study	501	Smoking	use of cigarettes	Fecundability ratio; the monthly conception rate among exposed relative to unexposed	0.53 (0.33 ; 0.85)
Somigliana et al. 2016	Prospective case-control study	146	Diet	Concentration of 25(OH)D <20 ng/ml	Odds ratio of longer time to pregnancy	0.84 (0.42 ; 1.66)
Wesselink et al. 2016	Prospective cohort study	1318	Caffeine	≥300 mg caffeine / day	Fecundability ratio; the monthly conception rate among exposed relative to unexposed	1.15 (0.90 ; 1.48)
Wise et al. 2010	Prospective cohort study	1410	BMI	BMI 25-29 kg/m2 BMI 30-34 kg/m2 BMI ≥35 kg/m2	Fecundability ratio; the monthly conception rate among exposed relative to unexposed	0.72 (0.58 ; 0.90) 0.60 (0.42 ; 0.85) 0.48 (0.31 ; 0.74)
Wise et al. 2012	Prospective cohort study	3027	Ph. activity	≥5 hrs / week vigorous activity ≥5 hrs / week moderate activity	Fecundability ratio; the monthly conception rate among exposed relative to unexposed	0.68 (0.54 ; 0.85) 1.18 (0.98 ; 1.43)
Wise et al. 2013	Prospective cohort study	2022	BMI	BMI ≥35 kg/m2	Fecundability ratio; the monthly conception rate among exposed relative to unexposed	0.73 (0.61 ; 0.87)

Note: BMI = body mass index, Ph. activity = Physical activity

Table 4. Description and summary of data for 14 studies that investigated associations between lifestyle factors and first-trimester miscarriage.

Author	Study design	Sample size	Exposure	Outcome description	Outcome definition	OR (95% CI)
Andersen et al. 2015	Prospective cohort study	1683	Vitamin use	Concentration of 25(OH)D of <50 vs ≥ 50 nmol/L	Hazard ratio for miscarriage	2.50 (1.10 ; 5.69)
Cnattingius et al. 2000	Retrospective case-control study	1448	Caffeine Smoking	Among non-smokers; 100-299 mg of caffeine / day 300-499 mg of caffeine / day ≥500 mg of caffeine / day Smokers compared to non-smokers	Odds ratios for miscarriage	1.8 (1.2 ; 2.7) 2.7 (1.7 ; 4.5) 4.1 (2.1 ; 8.1) 1.5 (1.1 ; 2.1)
Feodor Nilsson et al. 2014	Retrospective cohort study	88373	Alcohol Caffeine Ph. activity	>4 alcoholic drinks per week drinking 0,5 - 7,5 cups of coffee / day drinking >8 cups of coffee / day 61-120 minutes / week regular ph. activity 121-180 minutes / week 181-300 minutes / week >300 minutes / week	Hazard ratio for miscarriage	2.81 (2.25 ; 3.50) 1.28 (1.14 ; 1.42) 2.23 (1.79 ; 2.78) 1.83 (1.57 ; 2.13) 2.06 (1.72 ; 2.47) 2.47 (2.07 ; 2.93) 3.29 (2.71 ; 3.99)
Gaskins et al. 2014	Prospective cohort study	11072	Folic acid	Folate supplement use ≥1000 mcg / day, fetal loss <8 wks Folate supplement use ≥1000 mcg / day, fetal loss 8-11 wks	Relative risk of miscarriage	0.79 (0.64 ; 0.97) 0.76 (0.63 ; 0.92)
Gaskins et al. 2016	Retrospective cohort study	27580	Alcohol	>10 grams of alcohol / day, miscarriage < 8 weeks >10 grams of alcohol / day, miscarriage 8-11 weeks	Relative risk of miscarriage	1.09 (0.92 ; 1.30) 1.02 (0.86 ; 1.22)
Hahn et al. 2014	Prospective cohort study	5132	BMI	BMI ≥ 30 kg/m2	Hazard ratio for miscarriage	1.34 (1.01 ; 1.77)
Hahn et al. 2015	Prospective cohort study	5132	Caffeine	>300 mg caffeine per day (preconceptionally)	Hazard ratio for miscarriage	0.93 (0.72 ; 1.22)
Kesmodel et al. 2002	Prospective cohort study	18226	Alcohol	5 alcoholic drinks per week	Hazard ratio for miscarriage	3.7 (2.0 ; 6.8)
Parazzini et al, 1991	Retrospective case-control study	270	Alcohol BMI Caffeine Smoking	Alcohol consumption in pregnancy BMI ≥ 22.5 kg/m2 Coffee consumption in pregnancy Current smoking in pregnancy increasing number of cigarettes / day	Relative risk of recurrent miscarriage	0.9 (0.6 ; 1.5) 1.1 (0.6 ; 2.0) 1.4 (0.7 ; 2.6) 1.4 (0.8 ; 2.9) p for trend 0.04

Ronnenberg et al, 2002	Prospective case-control study	458	Folic acid Vitamin use	lowest quintiles of plasma folate concentration (≤ 6.60 nmol/L) lowest quintiles of plasma Vitamin B6 concentration (≤28.9 nmol/L)	Odds ratios for miscarriage	1.5 (0.6 ; 3.8) 2.5 (0.8 ; 7.8)
Strandberg-Larsen et al, 2008	Prospective cohort study	89201	Alcohol	binge drinking in first 16 weeks of pregnancy	Hazard ratio for miscarriage	0.84 (0.62 ; 1.14)
Windham et al, 1997	Prospective cohort study	5307	Alcohol	>3 alcoholic drinks per week	Odds ratios for miscarriage	2.3 (1.1 ; 4.5
Xu et al, 2014	Retrospective case-control study	1860	Alcohol BMI Diet Ph. activity Smoking Vitamin use	>4 times per week alcohol consumption Pre-pregnancy BMI ≥ 30 kg/m2 eating fresh fruit / vegetables daily >2 times per week, ≥0.5 hr smoking >20 cigarettes per day during first 12 weeks of pregnancy vitamin supplement use	Odds ratios for miscarriage	1.04 (0.79 ; 1.27) 1.05 (0.89 ; 1.25) 0.86 (0.49 ; 1.22) 0.72 (0.51 ; 0.88) 1.59 (1.12 ; 3.16) 0.75 (0.49 ; 0.91)
Zhou et al, 2016	Prospective cohort study	2940	BMI	Pre-pregnancy BMI <18.5 kg/m2 Pre-pregnancy BMI 24 - 27.9 kg/m2 Pre-pregnancy BMI ≥28 kg/m	Relative risk for miscarriage	2.57 (1.35 ; 4.89) 2.45 (1.26 ; 4.77) 2.84 (2.84 ; 6.57)

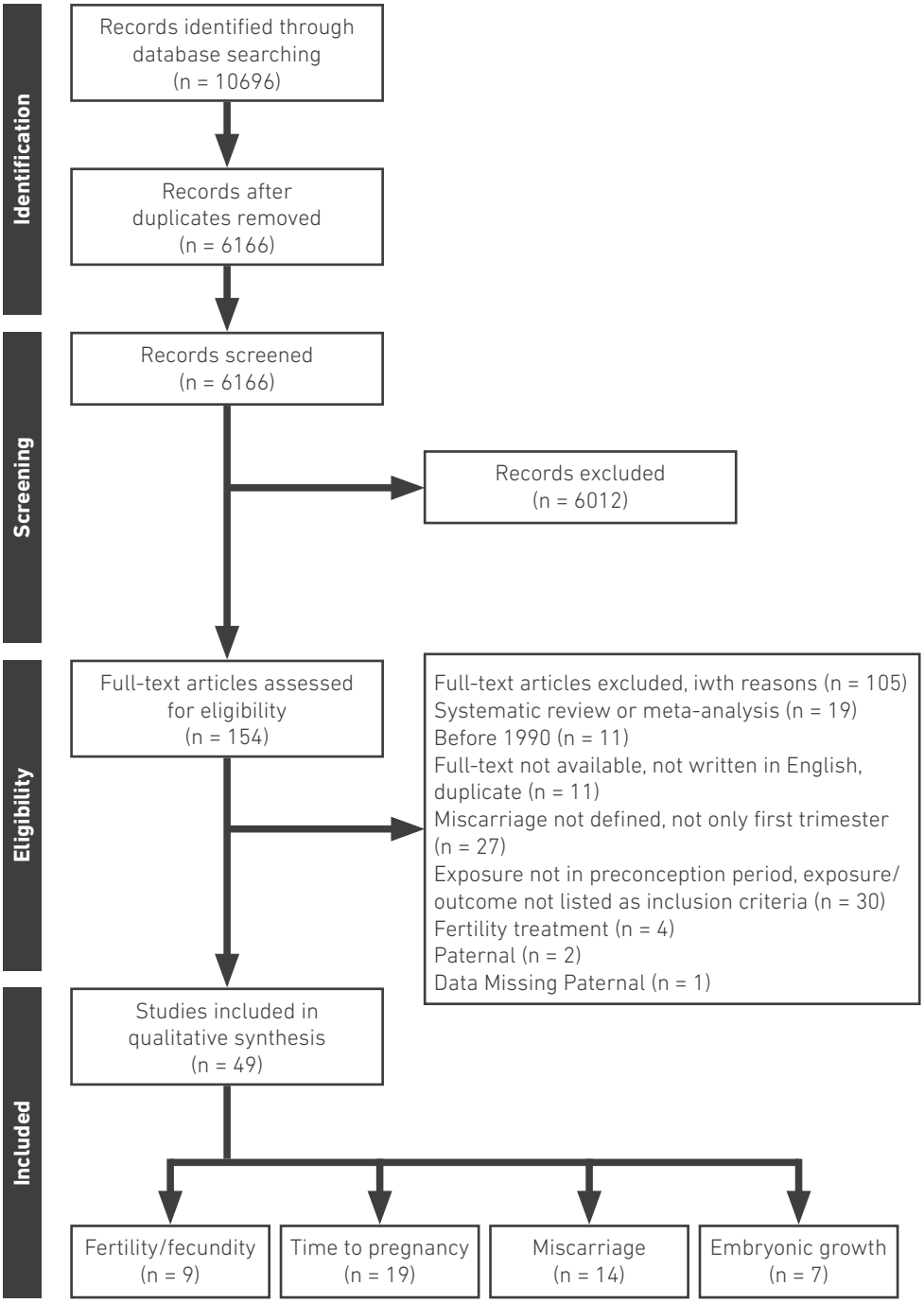
Note: BMI = body mass index, Ph. activity = Physical activity

Table 5. Description and summary of data for 7 studies that investigated associations between lifestyle factors and embryonic growth.

Author	Study design	Sample size	Exposure	Outcome description	Outcome definition	Effect estimate (95% CI)
van Uitert et al. 2013	Prospective cohort study	87	Alcohol BMI Folic acid Smoking	Periconception alcohol use BMI kg/m2 moment of initiation of folic acid; post conception Periconception smoking ≥10 cigarettes per day	CRL difference (mm)	-0.05 (-0.069 ; -0.017) 0.095 (-0.11 ; 0.17) 0.27 (-0.311 ; 0.49) -0.46 (-0.64 ; -0.077)
van Uitert et al. 2014	Prospective cohort study	440	Folic acid	Quartile 1 (814-1223 nmol/L) Quartile 2 (1224-1512nmol/L) Quartile 4 (1813-2936 nmol/L)	CRL difference (mm)	-0.49 (-0.66 ; -0.2) -0.45 (-0.64 ; -0.14) -0.54 (-0.7 ; -0.3)
Bakker et al. 2010	Prospective cohort study	1310	Caffeine	>6 units of caffeine per day	CRL difference (mm)	4.54 (-8.99 ; -0.09) p for trend <0.05
Bouwland-Both et al. 2013	Prospective cohort study	847	Diet	high adherence to an energy-rich dietary pattern	CRL difference (mm)	1.62 (0.52 ; 2.72) p for trend <0.05
Mook-Kanamori et al. 2010	Prospective cohort study	1631	Alcohol BMI Folic acid Smoking	alcohol consumption compared to no consumption per 1 SD (4.08 units) increase in BMI No use of folic acid supplement smokers compared to non-smokers	CRL difference (mm)	0.40 (-0.31 ; 1.11) -0.01 (-0.35 ; 0.33) -1.33 (-2.41 ; -0.24) -0.98 (-1.79 ; -0.16)
Parisi et al. 2017	Prospective cohort study	234	Vitamin use	Vitamin B12 concentration of -2 SD (73.4 pmol/L) Total Homocysteine concentration of +2 SD (10.4 µmol/L)	delay in Carnegie stage (days)	1.4 (1.3 ; 1.4) 1.6 (1.5 ; 1,7)
Prabhu et al. 2010	Prospective cohort study	903	Smoking	smokers compared to non-smokers	CRL difference (mm)	0.23 (-0.23 ; 0.70)

Note: BMI = body mass index. CRL = crown-rump length

Figure 1. Prisma flowchart of in- and excluded studies



Supplemental Table 1. List of keywords

Keyword	Category
Diet	Exposure
Smoking	Exposure
Alcohol	Exposure
Drugs	Exposure
Folic acid supplement use / Folate	Exposure
Multivitamin supplement use	Exposure
Lifestyle intervention	Exposure
Physical activity	Exposure
Body mass index (BMI) / Obesity	Exposure
Embryonic growth	Outcome
Fertility	Outcome
Fecundity / fecundability	Outcome
Time to Pregnancy	Outcome
Miscarriage	Outcome, clinical
Yolk sac	Outcome, ultrasound
Crown-rump length (CRL)	Outcome, ultrasound

Supplemental Table 2. ErasmusAGE quality score form for systematic reviews adjusted for: The influence of maternal lifestyle factors on periconception outcomes: a systematic review of observational studies.

Original: ErasmusAGE, 24 June 2013.

This quality score can be used to assess the quality of studies included in systematic reviews and meta-analyses and is applicable to both interventional and observational studies. The score was designed based on previously published scoring systems (Carter et al, 2010 and the Quality Assessment Tool for Quantitative Studies). The quality score is composed of five items, and each item is allocated 0, 1 or 2 points. This allows a total score between 0 and 10 points, where 10 represents the highest quality.

The version presented below is a general version and needs to be adapted for each review separately , e.g. concerning what study size is large or small within the study field, what exposure and outcome measurement methods are adequate, and what the key confounders are. Decisions on these detailed criteria should be based on literature, guidelines and/or discussions with experts. The criteria should be defined before the review process.

Smoking

1. Study design

0 for studies with cross-sectional data collection

1 for studies with longitudinal data collection (both retrospective and prospective)

2 for intervention studies

2. Study size (predefined) *

Observational studies

0 small population for analysis: n < 1000

1 intermediate population for analysis: n = 1000-4999

2 large population for analysis: n > 5000

3. Exposure

Observational studies

0 if the study used no appropriate exposure measurement method or if not reported

1 if the study used moderate quality exposure measurement methods (self-reported)

2 if the study used adequate exposure measurement methods (real measurement)

4. Outcome

0 if the study used no appropriate outcome measurement method or if not reported

1 if the study used an appropriate outcome measurement methods (self-reported)

2 if the study used an appropriate outcome measurement methods (real measurement)

5. Adjustments

0 if findings are not controlled for at least for all three key confounders, as mentioned below† *

1 if findings are controlled for key confounders

2 if an intervention is adequately randomized or when findings are additionally controlled for at least two

* Needs to be specified for each review, based on literature, guidelines and/or expert opinions in the field

† Either adjusted for in the statistical analyses; stratified for in the analyses; or not applicable (e.g. a study in women only does not require controlling for sex)

Strong adherence to a healthy dietary pattern is associated with better semen quality, especially in men with poor semen quality.

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Elsje C. Oostingh
Régine P.M. Steegers-Theunissen
Jeanne H.M. de Vries
Joop S.E. Laven
Maria P.H. Koster

Abstract

Objective To study associations between periconceptional dietary patterns and semen quality parameters.

Design Prospective periconception cohort study.

Setting Tertiary hospital in the Netherlands.

Patients 129 male partners of pregnant women who participated in the Rotterdam Periconception Cohort (Predict study).

Interventions None.

Main outcome measures Semen quality parameters (ejaculate volume, sperm concentration, total sperm count, progressive motility, immotile sperm, total motile sperm count (TMSC)).

Results Men included in our study were on average 35 (SD ±6) years of age and had a Body Mass Index of 26.4 ± 4 kg/m^2. Two dietary patterns were identified using Principle Component Analysis, which were labeled as 'healthy' and 'unhealthy'. An increase of one factor score (stated as β) represents an increase of one SD. Sperm concentration (β=0.278, 95%CI 0.112;0.444), total sperm count (β =1.369, 95%CI 0.244;2.495), progressive motility (β=4.305, 95%CI 0.675;7.936) and total motile sperm count (β =0.319, 95%CI 0.113;0.526) were all positively associated with a strong adherence to the 'healthy' dietary pattern. Subgroup analysis showed that these associations were mainly present in men with a TMSC <10 million spermatozoa. Although there was a trend towards a diminution in semen quality, we found no significant associations with strong adherence to the 'Unhealthy' dietary pattern.

Conclusion The positive associations between strong adherence to a 'healthy' dietary pattern and semen parameters in men with poor semen quality support the importance of preconceptional tailored nutritional counseling and coaching of couples who are trying to conceive.

Introduction

Over the past decades, several studies have provided evidence that semen quality in humans might be decreasing, which might lead to an increase in male subfertility (130, 131). This can be caused by intrinsic factors, such as genetic- or congenital disorders and cancer, however, a decline in semen quality is also observed in healthy men without any medical history (131, 132). Besides intrinsic factors, semen quality can also be affected by modifiable lifestyle behaviors. For example, recent studies suggest that specific nutritional factors can affect semen quality (125, 133, 134).

Parental nutritional status is a crucial determinant of normal reproductive function (12) and the nutritional environment of the fetus affects future child health as stated by the Developmental Origin of Health and Disease (DOHaD) paradigm (135). Malnutrition is known to disturb several metabolic pathways, prominent among these is the one-carbon (1-C) metabolism (136, 137). The 1-C metabolism is in particular essential for DNA synthesis and phospholipid and protein biosynthesis. Derangements in this metabolism can lead to excessive oxidative stress which can detrimentally affect gametogenesis and fertilization (5, 138). Several nutrients serve as substrate or cofactor to support the 1-C metabolism, for example folate and vitamin B12 (5).

A low intake of full-fat dairy food, sweets and processed meat, as well as a high intake of folate-rich food sources, such as fruits and vegetables, have been positively associated with semen quality (133, 134, 139-141). A high fruit, vegetable, fish and whole grain intake is associated with less sperm DNA damage than usual (125). Moreover, the use of folic acid and zinc supplements is known to increase the total normal sperm count in subfertile men (142, 143). Despite these findings, the impact of a healthy or unhealthy dietary pattern remains unclear, because most studies have only focused on the associations between semen quality and single or only a few nutrients or food groups. This single nutrient approach may be limited since people consume meals that consist of a varying combination of foods containing lots of different nutrients (144, 145). Some other studies have used dietary pattern analysis, which is a more accurate approach in order to identify the effect of the balance between food groups and nutrients on semen quality (145-147).

During preconception counseling there is often little attention paid to nutrition, especially in men. However, since an unhealthy dietary pattern can be modified, couples planning a pregnancy may benefit from the knowledge of the possible positive effect of nutrition on semen quality. To substantiate this, we aimed to identify dietary patterns in men and study associations with semen quality parameters.

Materials and Methods

Study population

This study was part of the Rotterdam Periconceptional Cohort (Predict study), an ongoing prospective tertiary hospital-based cohort study embedded in patient care and conducted at the department of Obstetrics and Gynecology of the Erasmus University Medical Centre, Rotterdam, the Netherlands. The details of this study have previously been described elsewhere (29).

For the current study, we selected men of the participating couples who were included in the study before 12 weeks of gestation between November 2010 and November 2014. Men were excluded when a semen sample was not available and/or when there was no information about their dietary patterns based on a food frequency questionnaire (FFQ). The window between the date of semen analysis and the date on which the FFQ was completed was restricted to a maximum of one year, because of the accumulating evidence that dietary patterns remain reasonably constant over time, except for periods of dieting and illness (148-150). If there was more than one semen sample available, the parameters of the sample closest to the moment of completing the FFQ were used in this study (***Figure 1***). The study protocol has been approved by the Medical Ethical and Institutional Review Board of the Erasmus MC, University Medical Centre in Rotterdam, the Netherlands, and all male participants provided written informed consent (METC Erasmus MC 2004-277).

Data collection

At study entry, all male participants completed a self-administered general questionnaire covering details on paternal age, ethnicity, educational level, medical history, previous children and periconceptional lifestyle (smoking, alcohol use, and

folic acid or multivitamin supplements use) at enrollment. At the same moment, qualified research nurses obtained anthropometric measurements (height, weight, waist-hip ratio, and blood pressure) (29). A validated semi-quantitative FFQ, developed by the division of Human Nutrition, Wageningen University, the Netherlands, was used to estimate habitual food intake over the four weeks before study entry (151, 152). The FFQ consists of 196 food items structured according to meal patterns, with questions including consumption frequency, portion size and preparation method. Intake of food, food groups, and energy and nutrients were determined using the Dutch food composition table (153). At enrollment, the research nurses checked the FFQs in a standardized manner for completeness and consistency.

Semen analysis

Semen samples were collected via masturbation into polypropylene containers. Within one hour, samples were liquefied, and the semen parameters semen volume, sperm concentration, total sperm count, percentage progressive motility, and percentage immotile motility were assessed according to World Health Organization (WHO) guidelines (World Health Organization, 2010). We defined normospermia as a total motile sperm count (TMSC) ≥10 million spermatozoa.

All semen analyses were performed by expertized laboratory staff at the Erasmus MC, University Medical Center Rotterdam, the Netherlands. Semen samples were not routinely collected as part of the Predict study, but only when there was a medical indication. Semen parameters were retrieved from medical records to obtain all the data required for our study.

Statistical analysis

First, we compared the baseline characteristics between men that were included and excluded in our study to investigate whether our study sample was a representative reflection of the entire Predict cohort (selection bias). These characteristics were expressed as medians with interquartile ranges (IQR) or absolute numbers with percentages and compared using either Mann-Whitney U or Chi-square tests.

A total of 196 food items from the FFQs were reduced to 23 predefined food groups based on origin and similar nutrient content (144). Next, we performed a Principal Component Analysis (PCA) to identify dietary patterns based on the correlation among food groups (145). Practically, PCA is a standard multivariate statistical technique that aggregates specific food groups on the basis of the degree to which food items are reciprocally correlated (145, 154). When PCA is performed, a factor loading is automatically calculated for each food group, showing the extent to which each food group is correlated with the specific dietary pattern. Finally, each person automatically receives a score to represent their adherence to every dietary pattern. Our PCA resulted in the identification of 23 dietary patterns (principal components). In order to reduce the bias of multiple testing and to identify the most common used dietary patterns in the study population, we selected two dietary patterns, with Eigenvalues >2.0 for investigation of the associations with the semen parameters. Additionally, the nutrient intake in men with strong (positive correlation) or weak (negative correlation) adherence to these patterns was calculated and compared using Mann-Whitney U tests.

Continuous semen parameters (volume, count and concentration) were log transformed to obtain normal distributions. Linear regression analysis was performed to establish associations between the factor score of each dietary pattern as independent variables and the semen parameters as dependent variables. An increase of one factor score (stated as β) is equal to an increase of one standard deviation and can thus be interpreted as such. First, we performed a crude analysis that was only adjusted for energy intake. Next, we constructed an adjusted model in which we additionally corrected for potential confounders that were based on study population and literature (i.e. paternal age, BMI, ethnicity, educational level, smoking, and alcohol use). Finally, we performed a similar multivariable linear regression analysis among subgroups of men with a TMSC below or above 10 million spermatozoa. P-values < 0.05 were considered statistically significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software for Windows (version 21.0, IBM SPSS, Statistics for Windows, Armonk, NY: IBM corp).

Results

A total of 1,435 men participated in the Predict study. After excluding men who did not match our inclusion criteria, 129 men were further analyzed (***Figure 1***). Baseline characteristics of included and excluded men are shown in ***Table 1***. Men included in our study were on average older (36 vs. 34 years, p=0.001), a higher percentage used folic acid supplements (21.7% vs 6.9% , p<0.001), and a higher percentage conceived by In Vitro Fertilization (IVF) / Intracytoplasmatic Sperm Injection (ICSI) treatment (59.7% vs 13.0%, p<0.001). Also, these men seemed to have slightly lower TMSC compared to the men that were excluded. All other characteristics did not significantly differ between groups. Semen parameters of our study group, stratified by TMSC below and above 10 million spermatozoa, are shown in ***Supplemental table 1***.

The two dietary patterns (selected from a total of 23 patterns) identified by PCA explained 23.1% of the variance of the overall dietary intake of the total study population. The first pattern was labelled 'unhealthy', because of high intakes of dairy, mayonnaise, margarines, sauces, snacks and sweets, and explained 12.2% of the total variance. The second pattern was labelled 'healthy', because of high intakes cereals, fruits, legumes, vegetables, and olive oil that explained 10.9% of the total variance (***Table 2***). ***Supplemental table 2*** shows the macro- and micronutrient intakes of men with strong or weak adherences to the 'healthy' and 'unhealthy' dietary pattern. Men with a strong adherence to the 'healthy' dietary pattern have a higher vitamin intake and slightly higher intakes of healthy fats (polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA), docosaheaxaenoic acid (DHA)) compared with men who have a weak adherence to this pattern or a strong adherence to the 'unhealthy' dietary pattern.

After adjustment for potential confounders, a strong adherence to the 'healthy' dietary pattern was positively associated with total motile sperm count (β=0.319, 95%CI 0.113;0.526), sperm concentration (β=0.278, 95%CI 0.112;0.444), sperm count (β=1.369, 95%CI 0.244;2.495) and progressive motility (β=4.305, 95%CI 0.675;7.936). In a subgroup analysis, these positive associations remained, but were only statistically significant in men with TMSC <10 million spermatozoa (***Table 3***). Strong adherence to the 'unhealthy' dietary pattern showed a trend towards diminution in total motile sperm count, sperm concentration, sperm count, progressive motility and immotile sperm, however these results were not statistically significant (***Table 3***).

Discussion

We identified two dietary patterns among men of couples visiting a tertiary hospital in early pregnancy and observed that high adherence to a 'healthy' dietary pattern is positively associated with semen quality, in particular in men with a TMSC <10 million spermatozoa. Furthermore, a trend of diminution of semen quality was seen in men with a high adherence to an 'unhealthy' dietary pattern.

These results confirm our previous findings in which only male partners of women undergoing IVF or ICSI treatment were included. In that study it was shown that a high adherence to a 'health conscious' pattern resulted in a lower DNA Fragmentation Index (DFI) as well as a higher percentage of progressive motility and thus better semen quality (125). The study of Jurewicz et al showed that high adherence to a 'prudent pattern', representing high intakes of fish, chicken, fruits, cruciferous vegetables, tomatoes, leafy green vegetables and whole grains, increases semen quality, reflected by a higher sperm concentration and lower DFI, and the testosterone level (134). Recently, the same authors also observed a positive effect of strong adherence to the prudent pattern in men and a decrease in chromosomal disorders in offspring (155). These studies have in common that in particular high intakes of fruits, vegetables and legumes are associated with a higher sperm concentration, whereas we also demonstrated positive effects on sperm count, total motile sperm count and percentage progressive motility.

The positive effects of high adherence to a 'prudent pattern' is further substantiated by Gaskins et al showing a positive and significant association with an increased percentage of progressive sperm motility (156). In that study the authors only examined healthy adolescent men, while in our study there was a larger variety of ages, which is more applicable to the general reproductive male population. Other studies of the same group also showed that a higher total fat intake was negatively associated with total sperm count, sperm concentration and sperm morphology and positively with asthenozoospermia (32,33,34). These associations appeared to be driven primarily by the intake of saturated fats derived from dairy (33). This is substantiated by our results showing that the intake of dairy, but also of other saturated fat sources as mayonnaise, snacks and sauces, was low in men with strong adherence to the 'healthy'

dietary pattern. On the other hand the intake of 'healthy fats' such as PUFA, EPA and DHA was higher in men who strongly adhered to the 'healthy' dietary pattern. The positive effect of these compounds of the 'healthy' dietary pattern on semen quality can be explained by the high contents of spermatozoa, which play a crucial role in fertilization (157). In addition, the intake of trans fatty acids (TFA) from largely the same sources, e.g. snacks, mayonnaise and other sauces, is also known to negatively influence semen quality (158, 159). We observed that the contribution of sugars was high in the unhealthy dietary pattern, which is supported by the inverse association shown between sugar intake and sperm motility among young healthy men (160). These findings all support the trends of positive and negative associations between 'healthy' and 'unhealthy' dietary patterns, respectively, and semen parameters of men in our study sample.

Micronutrients such as vitamins and trace metals also play an important role in normal testicular development, spermatogenesis and sperm motility. For example, seminal plasma zinc concentrations differ significantly between fertile and subfertile men and total normal sperm count increases after supplementation of folic acid and zinc sulfate (141, 143, 161). Both groups of micronutrients are present in in particular in cereals, legumes and fruits, the food groups that were highly represented in our 'healthy' dietary pattern. Vitamin C and E also play a role in male fertility (162-164). These vitamins can be found in fruits and vegetables, the same food groups that strongly contribute to our 'healthy' dietary pattern. These findings are supported by negative associations between low intakes of fruits and vegetables and the risk of oligoasthenoteratospermia (15), and high intakes among the same food groups and a decreased risk of oligoasthenoteratospermia (14). The positive association between a healthy diet and semen quality was more pronounced in men with poor semen quality (i.e. TMSC <10 million spermatozoa). It is not likely to believe that the influence of nutrition on semen quality differs between fertile and subfertile men. However, in men with poor semen quality the effect sizes may be larger because the range of TMSC is much smaller and therefore it is very likely that there is more room for improvement compared to men with a TMSC ≥10 million spermatozoa (17). Moreover, the identified non-significant positive associations between dietary patterns and semen quality in men with TMSC ≥10 million spermatozoa, may be hampered by our sample size.

Strengths of our study are, first, the availability of validated FFQs as this is a comprehensive method to obtain detailed information about the habitual intake of foods and, second, the use of principal component analysis (PCA), as state-of-the-art method to identify dietary patterns (145, 154, 165). Besides, all semen analyses were performed in the same laboratory which increases the internal validity of the data. However, our study also has some limitations. For only a small proportion of men, both semen analyses and FFQ data were available. Semen analyses were only performed when there was a medical indication, and not routinely as part of the Predict study, so they had to be retrospectively matched to our cohort. Therefore, the time window between completing the FFQ and the semen analysis was relatively large for some men. Because dietary patterns have shown to remain largely the same over a period of time, we believe that this still provides us with accurate information on the association between periconceptional nutritional status and semen quality (148-150). An expected consequence of the fact that the semen analyses were performed by indication is that our study sample consists of a higher proportion of subfertile men (***Table 1***). Nevertheless, 26.4% of all pregnancies were spontaneously conceived and for the remaining pregnancies subfertility could also have been due to female factors.

Knowledge about the associations found in our study can be used in routine preconception counseling. This counseling can be a valuable tool to identify and reduce modifiable health risks in couples who are trying to conceive (166). Since only a small proportion of couples make use of preconception care, more public awareness is necessary. In our modern digital era, mobile Health (mHealth) or eHealth tools, such as the online coaching platform Smarter Pregnancy, may contribute to this awareness and stimulate couples to change and maintain healthy dietary patterns and lifestyle choices (25).

Conclusion

In conclusion, here we demonstrate that a strong adherence to a 'healthy' dietary pattern is positively associated with semen parameters, especially in men with low TMSC. Herewith we support findings from other studies and emphasize the importance of making sure that every couple planning a pregnancy is aware of the considerable effect of nutrition also on male fertility. To create this awareness, we recommend that counseling on dietary patterns, for both women and men, should be implemented in preconception care worldwide.

Acknowledgements

We thank Mr. S.P. Willemsen for his assistance with the statistical analysis and the Predict research team for their contribution to the data collection.

Figure 1. Flowchart of the source and included study population.

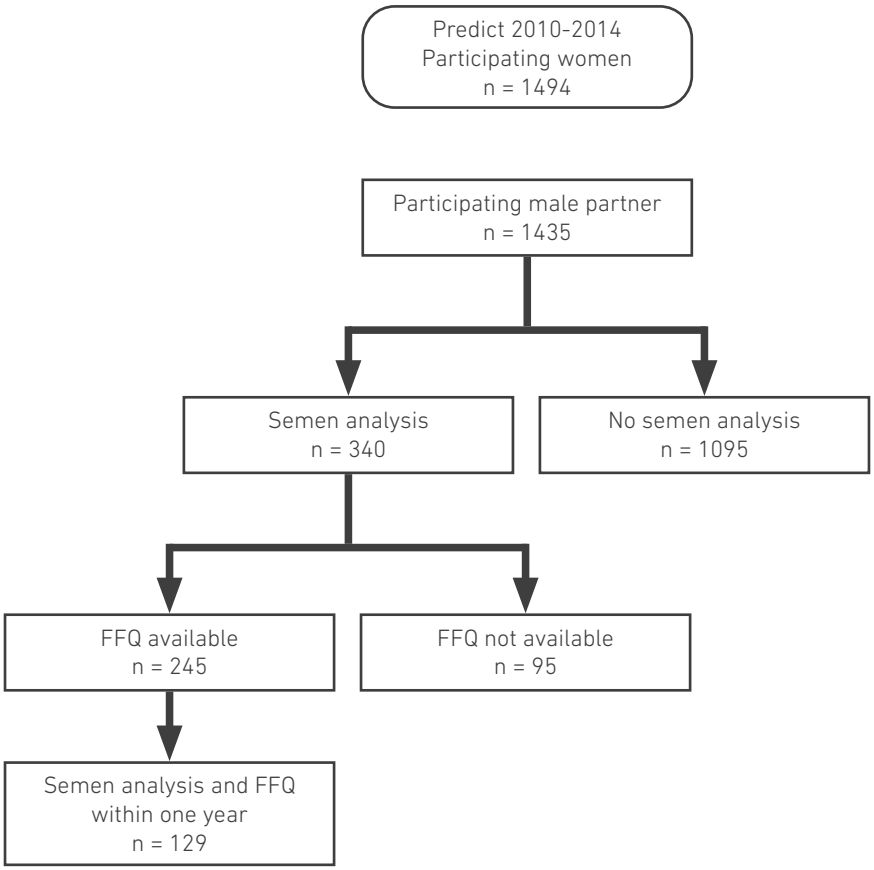

Table 1. Baseline characteristics of participants of the Predict cohort that were included and excluded in the current study.

	Included participants (n = 129)	Excluded participants (n = 1306)	p-value
Age (years)	36 (32-40)	34 (30-37)	0.001
BMI (kg/m2)	25.1 (22.9-27.3)	25.1 (23.3 - 27.7)	0.548
Geographic origin[a] n (%)			0.846
Western	109 (87.9)	748 (87.3)	
Non-western	15 (12.1)	109 (12.7)	
Missings	5	578	
Educational level[b] n (%)			0.369
High	60 (49.2)	399 (47.4)	
Intermediate	56 (45.9)	370 (43.9)	
Low	6 (4.9)	73 (8.7)	
Missings	7	593	
Lifestyle n (%)			
Smoking (yes)	31 (26.2)	252 (36.8)	0.568
Missings	11	622	
Alcohol (yes)	89 (74.8)	614 (89.8)	0.973
Missings	10	622	
Folic acid supplement use (yes)	26 (21.7)	46 (6.9)	<0.001
Missings	9	643	
Mode of Conception			<0.001
Spontaneous	34 (26.4)	745 (80.1)	
Hormone treatment / IUI	18 (13.9)	64 (6.9)	
IVF / ICSI	77 (59.7)	121 (13.0)	
Missings	0	376	
Semen parameters			
Ejaculate volume (ml)	2.50 (1.70-3.90)	2.60 (2.00-4.00)	0.322
Sperm concentration (10^6 / ml)	28.0 (8.00-63.0)	37.0 (15.0-61.0)	0.222
Total sperm count (10^6 / ml)	70.5 (14.4-156)	103 (29.0-206)	0.029
Total motile sperm count (10^6 / ml)	28.9 (3.08-78.7)	40.3 (10.1-88.9)	0.058
Progressive motility (A+B) (%)	40 (25-51)	42 (29-52)	0.557
Immotile sperm (C+D) (%)	57 (46-72)	56 (46-66)	0.708
Missings	*0*	*1095*	

Values are expressed as median (interquartile range) or as number (%). BMI, body mass index; RBC, red blood cell; IUI, intrauterine insemination; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.

Table 2. Factor scores of 23 food groups within two dietary patterns identified through principle component analysis.

	Unhealthy pattern	**Healthy pattern**
Variance explained (%)	12.2	10.9
Alcohol	0.103	-0.147
Cereals	0.126	0.659
Dairy	0.742	0.007
Eggs	0.099	-0.111
Fish	0.110	0.071
Fruits	-0.140	0.206
Grains (refained)	-0.119	-0.009
Grains (whole wheat)	0.296	0.077
Legumes	-0.150	0.807
Liquid fat	-0.163	-0.114
Meat	0.067	-0.122
Mayonnaise	0.438	0.061
Margarines	0.266	-0.056
Nuts	-0.006	0.185
Non-alcohol	0.084	-0.149
Olive oil	-0.019	0.291
Potatoes	0.225	0.085
Soup	-0.117	-0.007
Snacks	0.324	0.060
Sauces	0.662	0.042
Sugars	0.648	-0.117
Solid fat	-0.102	-0.138
Vegetables	0.061	0.708

Factor scores are presented as correlation coefficients

Table 3. Associations between the two identified dietary patterns and semen parameters for the total sample as well as stratified by total motile sperm count (TMSC) < or ≥ 10 million spermatozoa.

		Total sample				**TMSC < 10**				**TMSC ≥ 10**			
		Unhealthy		Healthy		Unhealthy		Healthy		Unhealthy		Healthy	
		Crude	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**
Ejaculate volume	β	0.041	0.003	0.005	-0.003	0.121	0.267	-0.012	-0.025	-0.008	-0.121	-0.023	-0.018
	95% CI	-0.049 ; 0.131	-0.116 ; 0.121	-0.085 ; 0.096	-0.100 ; 0.093	-0.040 ; 0.282	0.069 ; 0.466	-0.258 ; 0.234	-0.286 ; 0.237	-0.114 ; 0.099	-0.260 ; 0.017	-0.121 ; 0.074	-0.128 ; 0.092
Sperm concentration	β	0.044	-0.007	0.229	0.278	0.094	0.055	0.384	0.474	-0.052	-0.030	0.037	0.049
	95% CI	-0.115 ; 0.203	-0.221 ; 0.207	0.075 ; 0.383	0.112 ; 0.444	-0.129 ; 0.318	-0.229 ; 0.339	0.069 ; 0.699	0.174 ; 0.773	-0.169 ; 0.064	-0.190 ; 0.131	-0.070 ; 0.144	-0.076 ; 0.173
Total sperm count	β	-0.040	-0.794	1.262	1.369	0.090	0.041	1.214	1.549	-0.461	-1.160	0.289	0.479
	95% CI	-1.064 ; 0.985	-2.176 ; 0.588	0.267 ; 2.257	0.244 ; 2.495	-0.541 ; 0.720	-0.800 ; 0.882	0.362 ; 2.065	0.687 ; 2.411	-1.404 ; 0.481	-2.455 ; 0.135	-0.579 ; 1.157	-0.538 ; 1.496
Total motile sperm count	β	0.039	-0.124	0.282	0.319	0.062	0.036	0.375	0.476	-0.071	-0.202	0.038	0.069
	95% CI	-0.155 ; 0.234	-0.387 ; 0.139	0.094 ; 0.471	0.113 ; 0.526	-0.135 ; 0.259	-0.218 ; 0.289	0.104 ; 0.645	0.219 ; 0.733	-0.192 ; 0.050	-0.360 ; -0.043	-0.073 ; 0.150	-0.058 ; 0.196
Progressive motility (A+B)	β	0.327	-2.081	3.683	4.305	-0.524	-0.208	4.659	6.763	-0.751	-3.101	0.156	0.864
	95% CI	-3.045 ; 3.700	-6.628 ; 2.465	0.372 ; 6.993	0.675 ; 7.936	-6.036 ; 4.989	-6.249 ; 5.833	-3.413 ; 12.732	-0.103 ; 13.629	-3.111 ; 1.609	-6.346 ; 0.145	-2.012 ; 2.324	-1.7015 ; 3.443
Immotile sperm (C+D)	β	-0.716	-0.044	-1.154	-0.566	-2.219	-5.941	3.871	4.554	0.751	3.101	-0.156	-0.864
	95% CI	-4.506 ; 3.073	-5.260 ; 5.172	-4.940 ; 2.632	-4.815 ; 3.682	-12.249 ; 7.811	-19.810 ; 7.928	-11.028 ; 18.770	-12.191 ; 21.298	-1.609 ; 3.111	-0.145 ; 6.346	-2.324 ; 2.012	-3.443 ; 1.715

Adjusted for total energy intake, body mass index, age, ethnicity and smoking

Supplemental table 1. Semen parameters of included men for the total sample as well as stratified by total motile sperm count (TMSC) < or ≥ 10 million spermatozoa.

	Total sample	**TMSC < 10**	**TMSC ≥ 10**
Semen parameters	**n = 129**	**n = 45**	**n = 84**
Ejaculate volume (ml)	2.50 (1.70-3.90)	2.30 (1.30-2.90)	3.00 (1.80-4.20)
Semen concentration (106 / ml)	28.0 (8.00-63.0)	3.55 (0.60-9.05)	49.0 (28.0-79.0)
Total sperm count (106 / ml)	70.5 (14.4-156)	7.04 (0.57-16.8)	135 (72.0-198)
Total motile sperm count (106 / ml)	28.9 (3.08-78.7)	0.94 (0.00-3.20)	68.7 (30.0-96.5)
Progressive motility (A+B) (%)	40 (25-51)	17 (2-30)	48 (39-55)
Immotile sperm (C+D) (%)	57 (46-72)	79 (60-93)	53 (46-61)

Values are expressed as median (interquartile range)

Supplemental table 2. Dietary intake on nutrient levels in men with a strong and weak adherence to the (un)healthy dietary patterns.

	Unhealthy dietary pattern			Healthy dietary pattern		
	Weak	**Strong**	**p-value**	**Weak**	**Strong**	**p-value**
Total fat	76.3 (61.6-96.0)	100 (84.9-123)	< 0.001	87.7 (66.4-104)	92.7 (73.6-122)	0.159
Saturated fatty acids	25.9 (20.6-30.6)	36.2 (27.5-41.7)	< 0.001	27.2 (21.2-37.2)	28.9 (25.7-40.4)	0.212
MUFA	27.2 (22.5-35.1)	36.0 (28.9-42.3)	< 0.001	29.0 (23.4-37.2)	33.8 (25.9-43.0)	0.100
PUFA	17.1 (13.8-22.7)	20.7 (16.3-26.4)	0.028	17.9 (14.7-23.8)	21.0 (15.1-25.1)	0.184
Linoleic	14.6 (11.6-19.4)	17.2 (13.4-22.2)	0.049	15.1 (12.2-20.2)	17.3 (12.6-21.5)	0.216
Alanine	2.01 (1.61-2.51)	2.42 (1.84-2.93)	0.022	2.08 (1.57-2.60)	2.18 (1.73-2.97)	0.134
Eicosapentaenoic acid	0.05 (0.03-0.09)	0.06 (0.04-0.12)	0.165	0.05 (0.03-0.08)	0.07 (0.04-0.13)	0.030
Docosahexaenoic acid	0.06 (0.02-0.11)	0.07 (0.04-0.16)	0.162	0.06 (0.03-0.11)	0.08 (0.04-0.17)	0.089
Cholesterol	173 (133-232)	222 (169-296)	< 0.001	182 (141-256)	216 (161-253)	0.164
Proteins	76.6 (61.7-91.0)	89.9 (78.6-108)	< 0.001	78.0 (64.6-94.0)	88.4 (78.5-108)	0.003
Carbohydrates	250 (200-308)	320 (278-373)	< 0.001	281 (212-342)	284 (247-353)	0.271
Fiber	24.0 (18.0-29.1)	26.9 (21.4-33.2)	0.034	21.7 (17.8-28.1)	29.1 (24.6-35.2)	<0.001
Pyridoxine (Vitamin B6)	1.68 (1.40-2.07)	1.88 (1.62-2.66)	0.007	1.67 (1.40-2.15)	1.88 (1.67-2.44)	0.018
Cobalamin (Vitamin B12)	3.28 (2.37-4.46)	4.46 (3.74-5.57)	< 0.001	3.80 (2.72-4.75)	4.31 (3.31-5.58)	0.043
Folate	281 (220-380)	325 (263-443)	0.019	275 (220-380)	338 (282-465)	0.001
Zinc	9.78 (7.81-11.1)	11.6 (10.1-13.9)	< 0.001	9.64 (7.88-12.4)	11.1 (10.1-13.9)	0.002
Retinoic acid (Vitamin A)	454 (275-635)	579 (413-766)	0.012	494 (352-677)	502 (326-854)	0.535
Thiamin (Vitamin B1)	0.99 (0.82-1.25)	1.18 (0.99-1.45)	0.001	0.98 (0.84-1.24)	1.25 (1.04-1.41)	0.001
Riboflavin (Vitamin B2)	1.14 (0.93-1.45)	1.61 (1.32-1.96)	< 0.001	1.24 (0.97-1.65)	1.41 (1.22-1.70)	0.016
Niacin (Vitamin B3)	18.6 (15.2-24.1)	21.6 (17.5-26.7)	0.022	18.3 (15.3-24.1)	21.7 (18.3-26.6)	0.023
Vitamin C	105 (72.9-146)	107 (83.2-130)	0.776	100 (76.8-125)	119 (87.3-156)	0.056
Vitamin E	12.9 (10.8-17.6)	15.4 (12.5-22.6)	0.005	13.7 (11.2-17.7)	16.3 (11.3-21.0)	0.109
Alcohol	4.14 (0.50-14.0)	6.64 (2.47-18.5)	0.074	5.20 (1.32-16.3)	4.14 (1.59-17.4)	0.650

Values are expressed as median (interquartile range)

No independent associations between preconception paternal dietary patterns and embryonic growth

the Predict Study

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Elsje C. Oostingh
Iris de Vos
Annelies C. Ham
Elske M. Brouwer-Brolsma
Sten P. Willemsen
Alex J. Eggink
Eric A.P. Steegers
Régine P.M. Steegers-Theunissen

Abstract

Background & aim Several studies show the importance of periconceptional maternal dietary patterns on human embryonic growth. Healthy paternal nutrition has been associated with better semen quality and fecundability, however, evidence on the impact on pregnancy outcome is limited. Therefore, the aim of this study was to investigate the association between preconception paternal dietary patterns and first trimester embryonic growth, in addition to maternal dietary patterns, using the parameters longitudinal crown-rump length (CRL) and embryonic volume (EV).

Methods A total of 638 couples were enrolled in the Rotterdam Periconceptional Cohort and received longitudinal three dimensional transvaginal ultrasound scans from 7+0 up to 12+0 weeks of gestation. Virtual reality software was used to perform offline measurements of the embryonic CRL and EV. Food frequency questionnaires (FFQ) were used to estimate habitual food intake in couples. Principal component analysis (PCA) was performed to identify paternal and maternal dietary patterns. Linear mixed models adjusted for potential confounders were applied to analyze associations between paternal and maternal dietary patterns and embryonic growth parameters.

Results Strong adherence to the "Nuts and Fruits" maternal dietary pattern was positively associated with CRL (β=-0.057, 95%CI 0.015;0.099). After full adjustment, a significant inverse association was observed between the maternal "Solid fat and Sugars" dietary pattern and embryonic volume (β=-0.026, 95%CI -0.051;-0.001) in strictly dated spontaneous pregnancies. Adding paternal dietary patterns to the model did not significantly impact the effect estimates.

Conclusion Besides the shown associations between maternal dietary patterns and embryonic growth, no significant independent effect of paternal dietary patterns on embryonic growth parameters could be established. The biological importance of paternal nutrition on semen quality, however, supports the need of periconceptional tailored nutritional counselling of couples trying to conceive.

Introduction

The global epidemic of overweight and obesity affects approximately 2 billion men and women aged over 18 years (167). A poor balance between nutrition and physical exercise is the main cause of overweight and obesity affecting short- and long-term health, but also reproduction.

The periconception period is a critical timespan in reproduction covering 14 weeks before up to 10 weeks after conception (5). Previous research shows that periconceptional parental characteristics and lifestyle factors influence embryonic growth. For example, higher maternal age and folic acid supplement use are associated with increased embryonic growth while periconception smoking and alcohol consumption are associated with decreased embryonic growth trajectories (18, 50). Moreover, paternal birthweight is positively associated with embryonic growth trajectories (168).

Current scientific evidence strongly suggests that nutrition during this early period of life influences gametogenesis, fertilization, embryogenesis and placentation with consequences for growth and development of the fetus, neonate and even health in later life (18). However, most evidence of the impact of periconceptional nutrition on fertility and pregnancy outcomes has been investigated in women. Strong adherence to a healthy-, Mediterranean- or one-carbon-rich dietary pattern have been associated with an increased chance of pregnancy and reduced risks of several congenital malformations (169-172).
Research on the involvement of paternal nutrition on reproduction has been mainly focused on semen quality parameters. A review by Salas-Huetos et al, showed that healthy nutrition contributes to improved semen quality, whereas high intake of red meat, processed meat, and caffeine were inversely associated with fecundability (173). This was substantiated by another review which also showed that the intake of trans and saturated fats was consistently related to poor semen quality (172).

We hypothesize that paternal nutrition is not only important for semen quality, but also for embryonic development as a consequence of contributions to epigenetic reprogramming and influencing nutritional behavior of the pregnant woman. This is relevant because a small embryo is associated with an increased risk of miscarriage,

congenital malformations, fetal growth restriction and even with features of cardiovascular- and metabolic diseases in childhood (18, 174, 175).

So far the evidence is limited to animal studies providing evidence for a transgenerational impact of the paternal diet on offspring health by showing that offspring of male mice fed a low protein diet or a folate deficient diet had compromised cardiovascular and metabolic functioning in later life (176, 177). One of the possible explanations is the influence of the preconception paternal diet on the epigenetic programming of male gametes and subsequent transmission to the embryo (178, 179)

Nowadays, in human studies embryonic growth and development can be investigated with high precision of crown-rump length (CRL) (***Figure 2a***) and embryonic volume (EV) (***Figure 2b***) measurements using transvaginal 3-dimensional ultrasound (3D-US) with virtual reality (VR) techniques (48, 180, 181).

From this background the aim of this study was to investigate whether periconceptional paternal dietary patterns contribute to embryonic growth independent of the influence of maternal dietary patterns.

Materials and Methods

Study population

The present study was embedded in the Rotterdam Periconceptional Cohort (Predict Study), an ongoing prospective hospital-based birth cohort study, conducted at the department of Obstetrics and Gynecology of the Erasmus University Medical Centre, Rotterdam, the Netherlands (25). The protocol was approved by the Central Committee on Research in The Hague and by the local Medical Ethical Committee of the Erasmus MC in Rotterdam. All participants were informed about the study and signed a written informed consent. Details of this study have previously been described elsewhere (25). For the current study, couples were recruited before 8+0 weeks of pregnancy. We selected pregnant women of at least 18 years of age and their male partners who were included in the study between November 2010 and October 2016 and were able to speak and write in the Dutch language. Couples were excluded when the pregnancy was conceived after sperm or oocyte donation, when ultrasound data was unreliable

due to incomplete embryonic measurements or technical problems, or when nutritional data was missing or unreliable (i.e. total energy intake < 500 kilocalories (kcal)/ day for women and < 800 kcal/day for men) (182) (***Figure 1***).

Pregnancy dating

For spontaneously conceived pregnancies, gestational age was determined by the first day of the last menstrual period (LMP) if there was a regular cycle (25-35 days). If the menstrual cycle was prolonged (32-35 days), gestational age was adjusted for the duration of the menstrual cycle. If the gestational age deviated more than six days from the measured CRL, or when the LMP was unknown, the first CRL measurement was used to determine gestational age. For pregnancies conceived through intrauterine insemination (IUI), gestational age was calculated using the LMP or insemination date plus 14 days. For pregnancies derived from in vitro fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI), gestational age was determined on the day of oocyte retrieval plus 14 days. For pregnancies derived from cryo-embryo transfer, gestational age was calculated as the day of the embryo transfer plus 17 or 18 days (25, 183).

Dietary assessment

Habitual food intake was assessed using a validated 166-item semi-quantitative food frequency questionnaire (FFQ), with a reference period of four weeks. This FFQ was designed to cover >96% of the absolute level of food intake and >95% of the between-person variability of each nutrient under study assessed in the DNFCS from 1998 (184). Participants answered questions relating to frequency by selecting answers ranging from 'never' to '6-7 days per week'. Portion sizes were estimated using natural portions and commonly used household measures. Average daily nutrient intakes were calculated by multiplying the consumption frequency by the portion size and nutrient content in grams as indicated in the most recent Dutch food composition table (2011) (185). Intake levels for energy, macronutrients, dietary fiber, and selected vitamins were validated (151, 186, 187).

Embryonic growth

To determine embryonic growth, transvaginal 3D-US scans were performed in the 7th, 9th, and 11th week of gestation (19, 50). The ultrasound scans were executed by experienced researchers using a 6-12 MHz transvaginal probe and using GE Voluson E8 equipment and 4D View software (General Electrics Medical Systems, Zipf, Austria). Subsequently, the 3D-US scans were stored as Cartesian (rectangular) volumes and transferred to the BARCO I-Space (Barco N.V., Kortrijk, Belgium) at the Department of Bioinformatics, Erasmus University Medical Centre, Rotterdam. V-scope software was used to perform offline measurements of the CRL and EV. The CRL was measured three times in three dimensions, the average of these three measurements was used for analysis. The EV was measured with a semi-automated volume measuring application based on gray-scale differences.

General data

At enrolment all participating men and women filled out a general questionnaire covering details on age, geographical background, level of education, obstetric and medical history and periconceptional lifestyle behaviors (smoking, use of alcohol, folic acid supplement use) which was thoroughly checked by an experienced research nurse. At primary visit anthropometric measurements were conducted to obtain data on height and weight and blood pressure (25).

Statistical analysis

First, we compared the baseline characteristics between included and excluded participants to investigate whether our study sample was a representative reflection of the entire Predict cohort. These characteristics are reported as medians with interquartile ranges or as absolute numbers with percentages. Mann-Whitney U and Chi-square tests were used to compare respectively continuous variables or categorical data.

Principal component analysis (PCA) was used to derive paternal and maternal dietary patterns separately from the nutritional data obtained from the FFQ. First, the 166 food items were reduced to 24 predefined food groups based on their origin and nutrient content (144). Thereafter, PCA was performed. High correlations between food groups

were identified and factor loadings were calculated for each food group, indicating the extent to which each food group contributes to the specific dietary pattern (145). Based on visual inspection of the scree plot, three dietary patterns were retrieved with eigenvalues >1.75). All participants received factor scores for each dietary pattern, which represented their adherence to the specific pattern.

Maternal dietary patterns were expected to show a stronger association with embryonic growth than paternal dietary patterns. Therefore, linear mixed model analyses were conducted to assess the additional independent effect of each paternal dietary pattern on embryonic growth in the presence of each maternal dietary pattern. An increase of one factor score (represented by the beta (β)) is equal to an increase of one standard deviation and can thus be interpreted as such. To start, a first analysis was performed, adjusting the maternal dietary factor scores for gestational age and maternal total energy intake (model 1). Thereafter, we additionally adjusted for maternal BMI, maternal age, maternal smoking, nulliparous and fetal gender (model 2), paternal BMI, paternal smoking (model 3), and lastly paternal dietary factor scores and paternal total energy intake (model 4). Subsequently, model 1 and model 2 were used to determine the association between maternal dietary patterns and embryonic growth. Model 3 and model 4 were used to determine the additional association between paternal dietary patterns and embryonic growth. Subsequently, model 3 and model 4 were compared using a likelihood ratio test.
As linear mixed model analyses require a normal distribution of the data, square root transformations of CRL data and third root transformations of EV data were performed. Analyses were performed in two subgroups; 1. strictly dated spontaneous pregnancies, defined as known first day of LMP and regular menstrual cycle, and 2. IVF/ICSI pregnancies.

P-values of $p \leq 0.05$ were considered significant. Data analyses within this project were performed using SPSS Statistics for Windows, Version 21.0 (IBM Corp. Armonk, NY) and R version 3.4.1 (The R foundation for Statistical Computing).

Results

Study population

A total of 1,331 couples participated in the Predict study. After excluding pregnancies who did not match our inclusion criteria, 638 pregnancies were further analyzed. This included 94 pregnancies conceived through IVF, 104 from ICSI, and 440 spontaneously conceived, whether or not in combination with IUI (***Figure 1***).

Paternal and maternal characteristics of included and excluded pregnancies are shown in ***Table 1***. Median age of included men was 34 (31-38) years and they had a median BMI of 25.9 (23.8-28.3) kg/m^2. No significant differences were observed between included and excluded men. However, included women had a significantly lower BMI (24.4 vs. 24.7 kg/m^2; p=.025), were more often from Dutch origin (82.9 vs. 77.7%; p=.027) and were more likely to consume alcohol in the periconceptional period (34.3 vs. 27.3%; p=.010) compared to excluded women. In addition, included pregnancies were more often conceived after IVF/ICSI treatment (31.0 vs. 26.9%; p<.001) compared to excluded pregnancies.

The three paternal dietary patterns retrieved from the PCA, explained 27.5% of the total variance of the dietary intake. Paternal factor loadings for these dietary patterns are presented in ***Table 2***. The first paternal dietary pattern explained 11.5% of the total variance and was labelled "Whole wheat grains and Vegetables", reflecting high intakes of whole wheat grains, vegetables, and margarine and could therefore be labelled healthy. The second paternal dietary pattern explained 8.5% of the total variance and was labelled "Sauces and Snacks", reflecting high intakes of alcohol, sauces, and snacks and could therefore be labelled unhealthy. The third paternal dietary pattern explained 7.4% of the total variance and was labelled "Refined Grains, Fish and legumes", reflecting high intakes of refined grains, legumes, and fish.

Linear mixed model analyses neither showed an additional independent effect of paternal dietary patterns on embryonic growth in spontaneous pregnancies (strictly and not strictly dated) nor IVF/ICSI pregnancies (***Table 3***). Additionally, we compared the likelihood of model 4 with the likelihood of model 3 using the likelihood ratio test. No significant differences were found between these two models, for CRL as well as EV, in

strictly dated spontaneous pregnancies (CRL: p = .964; EV: p = .953), not strictly dated spontaneous pregnancies (CRL: p = .175; EV: p = 0.676), and IVF/ICSI pregnancies (CRL: p = .379; EV: p = .306) (data not shown).

The three maternal dietary patterns retrieved from the PCA, explained 27.2% of the total variance in dietary intake. Maternal factor loadings for these dietary patterns are presented in ***Table 2***. The first maternal dietary pattern explained 11.0% of the total variance and was labelled "Nuts and Fruits", reflecting high intakes of fish, fruits, legumes, and nuts. The second maternal dietary pattern explained 8.7% of the total variance and was labelled "Whole wheat Grains and Margarine", reflecting high intakes of margarine, potatoes, and whole wheat grains. The third maternal dietary pattern explained 7.5% of the total variance and was labelled "Solid fat and Sugars", reflecting high intakes of sodas/preserved juices, sugars and solid fat.

Strong adherence to the "Nuts and Fruits" maternal dietary pattern was associated with a larger CRL of 0.0032 mm per unit of increase in factor score (β=0.057, 95%CI 0.015;0.099) (model 1) but this association was limited to strictly dated spontaneous pregnancies only (***Table 4***). After adjustment for maternal factors, linear mixed model analyses showed a significant decrease in EV when there is a high adherence to the "Solid fat and Sugars" maternal dietary pattern (β=-0.026, 95%CI -0.051;-0.001) (model 2) (***Table 4***). Transformation to the original scale showed that adherence to the "Solid fat and Sugars" dietary pattern was associated with a decrease in EV of 0.000018 cm3 per unit of increase in factor score. This inverse association was limited to strictly dated spontaneous pregnancies. Furthermore, high adherence to the "Nuts and Fruits" or "Whole wheat Grains and Margarine" dietary pattern showed a trend toward an increase in EV, although not significant (model 2) (***Table 4***). For not strictly dated spontaneous pregnancies and IVF/ICSI pregnancies no significant associations were found between maternal dietary patterns and EV.

Discussion

In the present study, no significant associations were shown between adherence to periconceptional paternal dietary patterns and embryonic growth independent of maternal dietary patterns in spontaneous pregnancies or IVF/ICSI pregnancies. For maternal dietary patterns, a significantly inverse association between the periconceptional maternal "Solid fat and Sugars" dietary pattern and embryonic volume was found in strictly dated spontaneous pregnancies only.

The absence of the association in the not strictly dated group pregnancy can be explained by the fact that dating is performed by using CRL thereby ignoring all (patho) physiological variation in embryonic growth. The most likely explanation that no independent associations were found between paternal dietary patterns and embryonic growth is that the maternal dietary pattern and other unmeasured factors predominate the potential impact of the paternal dietary pattern.

The contributions of the paternal diet to embryonic growth and offspring health have mostly been investigated in animal studies, providing evidence for a transgenerational impact by showing that offspring of male mice fed a low protein diet or a folate deficient diet had compromised cardiovascular and metabolic functioning in later life (177, 188). Although animal studies provide valuable insights in the mechanism underlying paternal inheritance, these studies can often not be extrapolated to humans.

Several other paternal characteristics however have been associated with embryonic growth and birth outcomes in humans. Van Uitert et al. found a positive association between paternal birth weight and embryonic growth in the first trimester of pregnancy among 81 Dutch men participating in the Rotterdam Periconception Cohort between 2009 and 2010. One point increase in paternal birthweight Z-score was associated with an increased CRL of 0.0019 $\sqrt{}$mm/day (168). Furthermore, paternal obesity has shown to affect fecundability and birth outcomes. Ramlau-Hansen et al. showed that overweight and obese men had an increased risk of subfecundity, defined as waiting time to pregnancy of more than 12 months, compared to normal weight men (OR 1.15 and 1.49, for overweight and obese respectively) (189). Additionally, in 305 couples undergoing assisted reproductive technology (ART) paternal obesity was associated

with a decreased chance of clinical pregnancy (25.8% obese vs. 42.9% normal weight) and live birth rates (22.6% obese vs. 41.3% normal weight) (190). The effects of paternal smoking have been studied by Morales-Suárez-Varela and colleagues (191). A total of 87,930 pregnancies were included from the population-based Danish National Birth Cohort. Paternal smoking was associated with a 10% higher risk of fetal deaths and a 46% higher risk of still births compared to non-smoking fathers.

Although we found no significant association between paternal dietary patterns and embryonic growth, there are many indications that paternal nutrition affects semen quality and fecundability (173). In the reviewed studies, high intakes of fish, shellfish, seafood, poultry, cereals, vegetables, fruits and low-fat dairy products were positively associated with semen quality. High intakes of red meat, processed meat, caffeine and tea were inversely associated with fecundability, including rates of fertilization, pregnancy, or miscarriage. Besides, Vujkovic et al. observed that couples (n=161) undergoing IVF/ICSI treatment with a higher adherence to the "Mediterranean" dietary pattern had a 40% increased probability of pregnancy (116). However, as paternal adherence to the "Mediterranean" diet and IVF/ICSI outcomes were not studied in absence of maternal adherence to the "Mediterranean" diet, no conclusions could be drawn about the single impact of paternal adherence to the "Mediterranean" diet and IVF/ICSI outcomes.

Although paternal dietary patterns have not been examined in relation to embryonic growth yet, Parisi et al. showed that maternal adherence to a 'high fish, and olive oil, low meat' dietary pattern has been associated with increased embryonic CRL and EV per unit of increase in factor score (183). Likewise, our results indicated an inverse association between high adherence to the maternal "unhealthy" dietary pattern and EV. These results suggest that the intake of healthy food groups, containing essential nutrients, is positively associated with embryonic growth, whereas the intake of unhealthy food groups, lacking essential nutrients, is inversely associated with embryonic growth. In conjunction, these associations are not only reflected in embryonic growth, but also in offspring health. A Dutch case-control family study showed that maternal high intakes of fish and seafood were associated with a 70% reduced risk of congenital heart diseases in offspring (169).

Although this study does not show significant associations, we still believe that based on the epigenetic influences of paternal conditions on the programming of male gametes and subsequent transmission to the embryo, the paternal dietary pattern can influence embryonic growth as well (178, 179). Therefore, as lifestyle behaviours of couples strongly correlate, it is important for clinical care that those who are contemplating pregnancy are aware and encouraged to adopt healthy behaviours. This is a responsibility of these women and men as well as for health professionals.

An important strength of our study is its longitudinal prospective design, in particular addressing the periconceptional period, the large number of included men and women, and the use of comprehensive questionnaires with detailed information about the participants. Moreover, the validated FFQ measured participant's habitual food intake of the previous four weeks, minimizing day to day variation in food intake, and the derived dietary patterns reflect the overall nutritional intake of participants covering a wide range of nutritional factors instead of investigating single nutrients. Finally, the longitudinal 3D ultrasound scans were analyzed using the BARCO I-Space and V-scope software, providing 3D holograms of the embryos. Consequently, embryonic growth could be measured with high accuracy and precision (180). The CRL was measured three times, of which the inter- and intra-observer agreement had been shown to be very high (48, 192). Furthermore, the 3D imaging technique enabled us to measure embryonic volumes and use it as a second parameter of embryonic growth. Embryonic volume has been proposed to be a predictive measure of embryonic growth restrictions (192, 193).

Some limitations of the study have to be addressed as well. The cohort is embedded in a tertiary hospital, which limits external validity of our study because of the higher proportion of high risk pregnancies compared to the general population. In addition, a large proportion of the pregnancies were conceived through IVF/ICSI, compared to the general Dutch population (31.0% vs. 2.5%) (194). Although,the growth trajectories of embryos conceived through IVF/ICSI pregnancies are comparable to embryos conceived spontaneously, analyses were performed separately for spontaneously conceived pregnancies and IVF/ICSI pregnancies to minimize confounding by mode of conception (19). Embryonic growth is highly dependent on gestational age and an

incorrect estimation of gestational age could have attenuated the potential association between paternal dietary patterns and embryonic growth. However, the differentiation of spontaneous pregnancies in strictly dated and not strictly dated spontaneous pregnancies reduces confounding by gestational age. A total of 535 pregnancies was excluded because of missing CRL and EV measurements, which was mainly due to not fully visualized embryos at the beginning of the study. As a result, the CRL and EV of these embryos could not be measured. It was not known whether larger embryos were more often missed than smaller embryos. If this was the case, it may have diluted potential positive associations between paternal dietary patterns and embryonic growth. Since the FFQ was self-administered and filled out in retrospect, it is susceptible for recall bias. Furthermore, the FFQ was only validated for women at reproductive age, not for men. There are some indications that bias related to social desirability and social approval differ for men and women, possibly leading to misclassification of dietary exposure (195, 196). However, other studies observed that fathers and mothers have qualitatively comparable dietary patterns (197, 198). By using a semi-quantitative FFQ, differences in portion size between men and women were taken into account. Finally, although we have adjusted for many covariates in our analyses, residual confounding cannot be excluded.

Conclusion

In this study no significant associations between adherence to periconceptional paternal dietary patterns and embryonic growth independent of the maternal dietary patterns could be shown. Previous studies however revealed associations between paternal nutrition and semen quality, fecundability, and offspring health, which can be explained by preconceptional epigenetic influences of the paternal dietary pattern on male gametes and subsequent transmission to the embryo. Therefore, we emphasize that more research, should be focused in particular on the influence of paternal dietary patterns on prenatal growth and pregnancy outcome. This will enhance the awareness of the importance of healthy nutrition of couples contemplating pregnancy and health care professionals beyond pregnancy as an investment for health of current and future generations

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Statement of authorship RST conceived, designed the study, contributed to all versions of the manuscript and supervised all aspects of the study. IdV, EO and AH conducted the statistical analyses. IdV, EO and AH drafted the first version of the manuscript. SW supported with the statistical analyses. All authors contributed to the critical revision of the manuscript and approved the final version.

Conflict of Interest Statement All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. No disclosures were reported.

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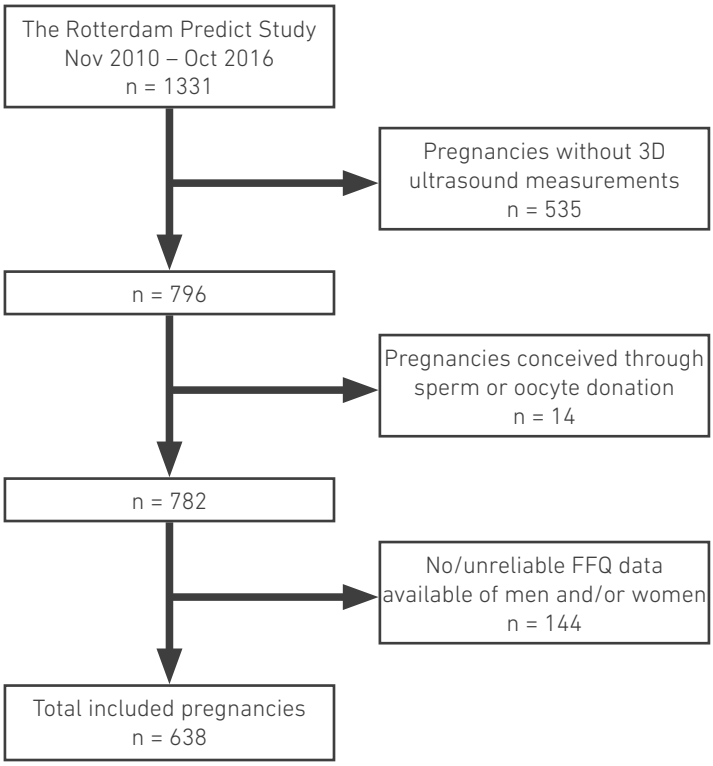
List of abbreviations

BMI: body mass index
CRL: crown-rump length
EV: embryonic volume
IUI: intrauterine insemination
IVF: in vitro fertilization
ICSI: intracytoplasmic sperm injection
LMP: last menstrual period
FFQ: food frequency questionnaire
3D-US: three dimensional ultrasound

Figure 1. Flowchart of the study population.



FFQ = Food Frequency Questionnaire, IVF = in vitro fertilization, ICSI = intracytoplasmic sperm injection.

Figure 2a. 3D measurement of crown-rump length (CRL), as depicted by the green line, of an embryo of 9 weeks of gestational age in the Barco I-space (with permission)

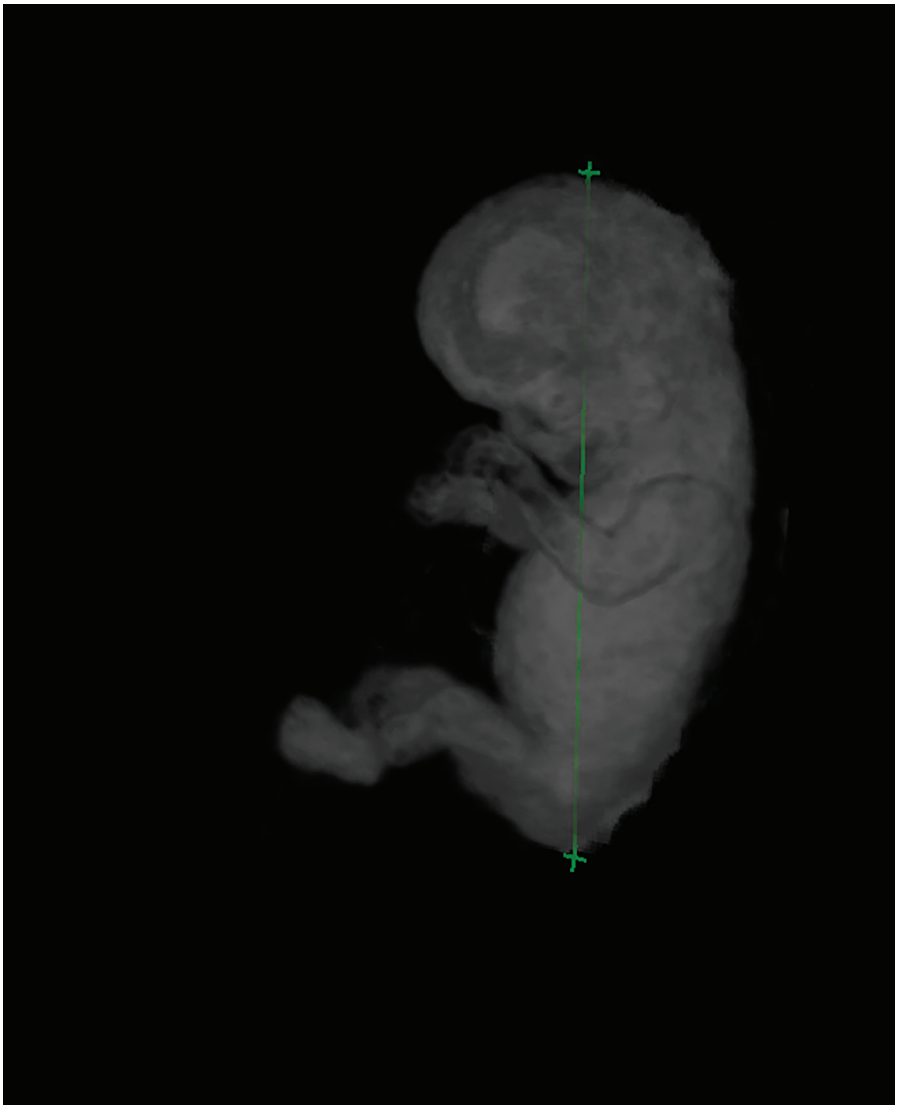


Figure 2b. 3D measurement of embryonic volume (EV) of an embryo of 9 weeks of gestational age in the Barco I-space (with permission)

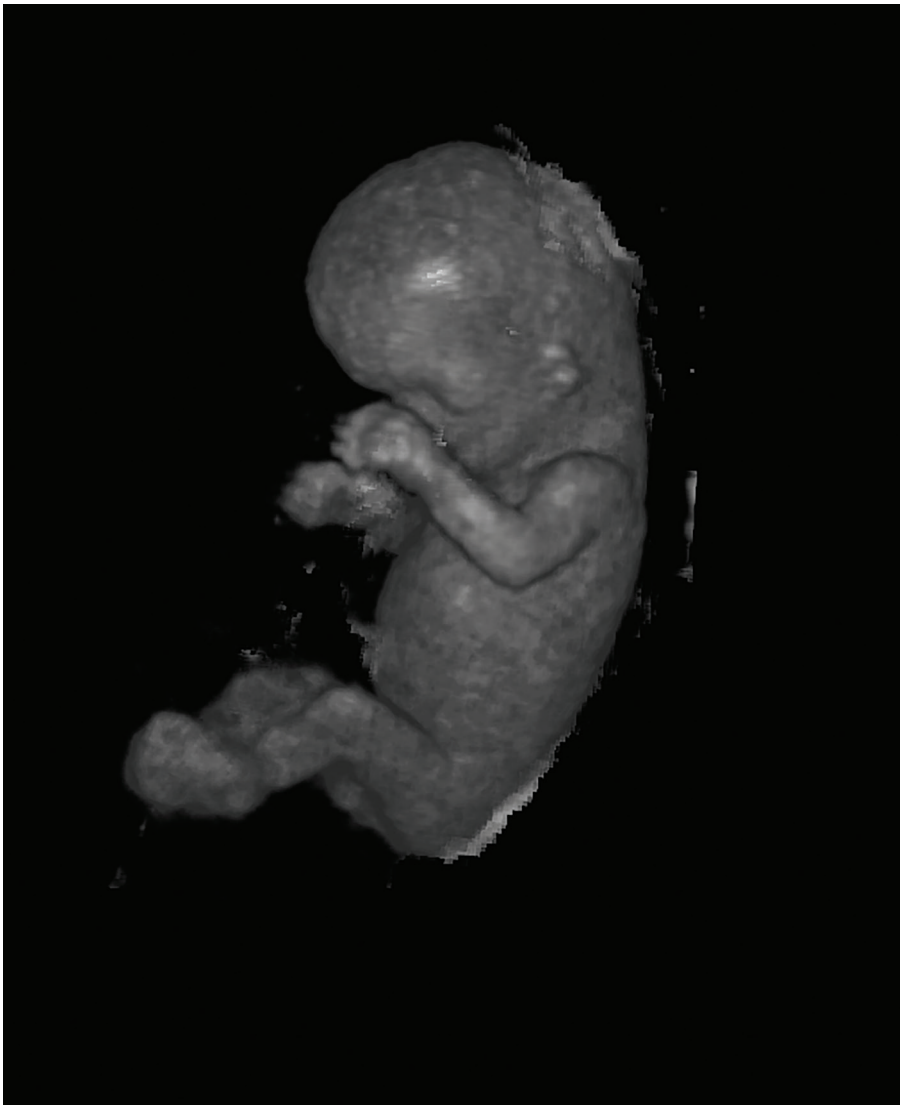


Table 1. Baseline characteristics of participants of the Predict cohort that were included and excluded in the current study.

	Paternal		Maternal	
	Included (n=638)	Excluded (n=693)	Included (n=638)	Excluded (n=693)
Age (years)	34 (31-38)	34 (31-38)	32 (29-35)	32 (29-35)
missings	104	247	51	55
BMI (kg/m2)	25.9 (23.8-28.3)	26.0 (23.8-28.3)	24.4 (21.9-28.0)*	24.7(22.3-29.1)
missings	77	213	46	53
Geographic background				
Dutch	519 (83.7%)	350 (85.0%)	520 (82.9%)*	421 (77.7%)
Other Western	23 (3.7%)	10 (2.4%)	31 (4.9%)	25 (4.6%)
Non-Western	78 (12.6%)	52 (12.6%)	76 (12.2%)	96 (17.7%)
missings	18	281	11	151
Education level				
Low	84 (13.6%)	62 (15.3%)	51 (8.2%)	51 (9.5%)
Intermediate	228 (36.8%)	140 (34.6%)	211 (33.7%)	209 (38.8%)
High	307 (49.6%)	203 (50.1%)	364 (58.1%)	279 (51.7%)
missings	19	288	12	154
Smoking (yes)	186 (30.7%)	117 (30.0%)	98 (15.7%)	90 (17.1%)
missings	32	303	14	168
Alcohol (yes)	455 (75.2%)	280 (72.0%)	214 (34.3%)*	144 (27.3%)
missings	33	304	15	166
Folic acid supplement use (yes)	49 (8.0%)	28 (7.2%)	616 (98.2%)	518 (97.2%)
missings	28	304	11	161
Multivitamin use (yes)	116 (19.2%)	74 (19.2%)	432 (69.0%)	392 (73.7%)
missings	33	308	12	161
Nulliparous (yes)	NA	NA	283 (46.5%)	236 (44.9%)
missings			29	167
Fetal gender (male)	NA	NA	311 (52.9%)	240 (50.8%)
missings			50	48
Mode of conception				
Spontaneous	NA	NA	440 (69.0%)*	122 (65.6%)
IVF/ICSI			198 (31.0%)	50 (26.9%)
Sperm/Egg donation			0 (0.0%)	14 (7.5%)
missings			0	507

Note: Data are represented as median with interquartile range (IQR) or as number with percentage.
BMI = Body Mass Index; IVF = In vitro fertilization; ICSI = Intracytoplasmic sperm injection.
* P ≤.05.

Table 2. Factor loadings of 24 food groups within three paternal and three maternal dietary patterns identified through principle component analysis.

	Paternal dietary patterns			Maternal dietary patterns		
Food groups	Whole wheat Grains and Vegetables	Sauces and Snacks	Refined Grains, Fish and Legumes	Nuts and Fruits	Grains and Margarine	Solid fat and Sugars
Variance explained (%)	11.5	8.5	7.4	11.0	8.7	7.5
Alcohol	-0.042	**0.646**	-0.137	-0.001	-0.058	-0.024
Cereals	0.225	-0.133	0.296	0.108	-0.118	-0.090
Dairy	0.210	0.073	0.102	-0.028	0.207	0.101
Eggs	-0.167	0.126	0.120	0.308	-0.355	-0.097
Fat	-0.158	0.111	-0.033	-0.048	-0.097	-0.022
Fish	0.009	0.098	**0.610**	**0.562**	-0.083	-0.075
Fruits	**0.418**	-0.254	0.212	**0.589**	0.118	0.285
Grains (refined)	-0.188	0.222	**0.626**	-0.073	-0.357	0.083
Grains (whole wheat)	**0.739**	-0.012	-0.199	0.163	**0.768**	-0.021
Legumes	0.052	-0.146	**0.549**	**0.578**	-0.150	-0.093
Liquid fat	0.045	-0.023	-0.019	-0.166	0.142	0.040
Margarine	**0.529**	0.164	-0.284	-0.208	**0.676**	-0.078
Mayonnaise	-0.055	**0.548**	0.189	0.123	0.077	0.112
Meat	0.151	0.366	-0.005	-0.021	0.222	0.004
Nuts	0.201	0.043	0.052	**0.649**	0.092	-0.027
Olive oil	**0.429**	0.231	0.228	0.049	0.071	0.109
Potatoes	0.324	0.113	-0.081	-0.045	**0.412**	0.388
Sauces	0.169	**0.594**	0.174	0.044	0.218	0.065
Snacks	0.044	**0.571**	-0.074	0.020	0.091	0.107
Solid fat	-0.045	-0.032	0.021	0.000	-0.114	**0.746**
Soup	0.127	0.208	-0.027	0.099	-0.020	0.093
Sugars	0.094	0.140	-0.157	-0.085	0.040	**0.738**
Sodas / Preserved Juices	-0.153	0.109	0.116	0.138	-0.034	**0.420**
Vegetables	**0.692**	0.041	0.216	0.367	0.278	0.302

Note: Factor loadings indicate the extent the food group is correlated to a specific dietary pattern and are presented as correlation coefficients. Factor loadings of significant relevance (>0.400 or <-0.400) are presented in bold.

Table 3. Effect estimates from the linear mixed model analysis for associations between paternal dietary patterns and embryonic crown-rump length (CRL) and embryonic volume (EV), stratified by strictly dated spontaneous pregnancies, not strictly dated spontaneous pregnancies, and IVF/ICSI pregnancies.

			Strictly dated spontaneous pregnancies (n=251)	**Not strictly dated spontaneous pregnancies** (n=139)	**IVF/ICSI pregnancies** (n=171)
Whole wheat Grains and Vegetables	CRL	β	-0.006	-0,048	-0.015
		95% CI	-0.069, 0.058	-0,095 ; -0,000	-0.061, 0.031
	EV	β	0.001	-0,015	-0.006
		95% CI	-0.022, 0.021	-0,036 ; 0,006	-0.025, 0.013
Sauces and Snacks	CRL	β	0.001	-0,015	0.006
		95% CI	-0.052, 0.052	-0,064 ; 0,034	-0.037, 0.050
	EV	β	-0.007	0,004	0.002
		95% CI	-0.025, 0.011	-0,017 ; 0,025	-0.016, 0.020
Refined Grains, Fish and Legumes	CRL	β	-0.005	-0,019	0.012
		95% CI	-0.051, 0.041	-0,060 ; 0,022	-0.030, 0.054
	EV	β	-0.003	-0,002	-0.010
		95% CI	-0.020, 0.014	-0,020 ; 0,017	-0.028, 0.008

Note: effect estimates (β) represent the amount of change in square root CRL ($\sqrt{}$/mm) and third root EV ($\sqrt[3]{}$/cm^3) per unit increase of the dietary factor score. For example for the 'Whole wheat grains and vegetables' dietary pattern; 1 unit increase in dietary factor score means an increase in CRL of $(-0.006)^2$ mm = 0.000036 mm
In this table, only the fully adjusted model 4 is presented (adjusted for maternal and paternal dietary factor scores, maternal and paternal total energy intake, maternal en paternal BMI, maternal and paternal smoking, maternal age, nulliparous and fetal gender)
IVF = in vitro fertilization, ICSI = intracytoplasmic sperm injection, CI = confidence interval

Table 4. Effect estimates from the linear mixed model analysis for associations between maternal dietary patterns and embryonic crown-rump length (CRL) and embryonic volume (EV), stratified by strictly dated spontaneous pregnancies, not strictly dated spontaneous pregnancies, and IVF/ICSI pregnancies.

			Model 1			**Model 2**			**Model 3**			**Model 4**		
			Strictly dated (n = 280)	**Not strictly dated** (n = 160)	**IVF/ICSI** (n=138)	**Strictly dated** (n = 257)	**Not strictly dated** (n = 147)	**IVF/ICSI** (n = 177)	**Strictly dated** (n = 251)	**Not strictly dated** (n = 139)	**IVF/ICSI** (n = 171)	**Strictly dated** (n = 251)	**Not strictly dated** (n = 139)	**IVF/ICSI** (n = 171)
Nuts and Fruits	CRL	β	0.057*	-0.008	0.005	0.057*	-0.008	0.005	0.057*	-0.008	0.005	0.057*	-0.008	0.005
		95% CI	0.015, 0.099	-0.045, 0.030	-0.025, 0.035	0.015, 0.099	-0.045, 0.030	-0.025, 0.035	0.015, 0.099	-0.045, 0.030	-0.025, 0.035	0.015, 0.099	-0.045, 0.030	-0.025, 0.035
	EV	β	0.014	0.008	-0.003	0.014	0.008	-0.003	0.014	0.008	-0.003	0.014	0.008	-0.003
		95% CI	-0.002, 0.030	-0.025, 0.010	-0.015, 0.010	-0.002, 0.030	-0.025, 0.010	-0.015, 0.010	-0.002, 0.030	-0.025, 0.010	-0.015, 0.010	-0.002, 0.030	-0.025, 0.010	-0.015, 0.010
Whole wheat Grains and Margarine	CRL	β	0.022	0.004	-0.009	0.022	0.004	-0.009	0.022	0.004	-0.009	0.022	0.004	-0.009
		95% CI	-0.021, 0.064	-0.032, 0.041	-0.041, 0.024	-0.021, 0.064	-0.032, 0.041	-0.041, 0.024	-0.021, 0.064	-0.032, 0.041	-0.041, 0.024	-0.021, 0.064	-0.032, 0.041	-0.041, 0.024
	EV	β	0.005	-0.016	-0.001	0.005	-0.016	-0.001	0.005	-0.016	-0.001	0.005	-0.016	-0.001
		95% CI	-0.012, 0.021	-0.032, 0.000	-0.013, 0.012	-0.012, 0.021	-0.032, 0.000	-0.013, 0.012	-0.012, 0.021	-0.032, 0.000	-0.013, 0.012	-0.012, 0.021	-0.032, 0.000	-0.013, 0.012
Solid fat and Sugars	CRL	β	-0.012	-0.012	-0.027	-0.012	-0.012	-0.027	-0.012	-0.012	-0.027	-0.012	-0.012	-0.027
		95% CI	-0.075, 0.050	-0.067, 0.042	-0.058, 0.005	-0.075, 0.050	-0.067, 0.042	-0.058, 0.005	-0.075, 0.050	-0.067, 0.042	-0.058, 0.005	-0.075, 0.050	-0.067, 0.042	-0.058, 0.005
	EV	β	-0.021	-0.013	0.002	-0.021	-0.013	0.002	-0.021	-0.013	0.002	-0.021	-0.013	0.002
		95% CI	-0.046, 0.0034	-0.038, 0.012	-0.012, 0.015	-0.046, 0.0034	-0.038, 0.012	-0.012, 0.015	-0.046, 0.0034	-0.038, 0.012	-0.012, 0.015	-0.046, 0.0034	-0.038, 0.012	-0.012, 0.015

Note: effect estimates (β) represent the amount of change in square root CRL (√mm) and third root EV (3√cm3) per unit increase of the dietary factor score. For example for the 'Nuts and Fruits' dietary pattern; 1 unit increase in dietary factor score means an increase in CRL of (0.057)2 mm = 0032 mm. * P ≤.05 IVF = in vitro fertilization, ICSI = intracytoplasmic sperm injection, CI = confidence interval

Model 1; adjusted for maternal dietary factor scores, total energy intake, and gestational age

Model 2; model 1 and additionally adjusted for maternal BMI, smoking, age, nulliparous and fetal gender

Model 3; model 2 and additionally adjusted for paternal BMI and smoking

Model 4; model 3 and additionally adjusted for paternal dietary factor scores and total energy intake.

Potential benefits of the use of sympathomimetics for asthmatic disease, on semen quality in men of subfertile couples

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Elsje C. Oostingh
Nicole A. Huijgen
Rivka Koedooder
Gert R. Dohle
Bruno H.C. Stricker
Régine P.M. Steegers-Theunissen

Abstract

Research question Is there an association between the use of sympathomimetics for asthmatic disease and semen quality in human?

Design Between 2007 and 2012 a prospective cohort study was conducted among couples visiting the preconception counselling clinic at a tertiary hospital in the Netherlands. Eight hundred eighty-two men of subfertile couples were included in this study and information on medication use was obtained from self-administered questionnaires. Moreover, data on semen parameters were retrieved from medical records.

Results The study population of men revealed a mean age of 34 ± 4 standard deviation (SD) years with a mean Body Mass Index (BMI) of 26.1 ± 2.3 SD kg/m^2, and sympathomimetic use was reported by 3.6%. The use of sympathomimetics was positively associated with a 10% higher sperm motility (β 10.265; 95% CI, 3.258-17.272) after adjustment for smoking, alcohol use, age, geographic background, BMI, folic acid supplement use, the four astronomical seasons and asthma/bronchitis. Subgroup analysis between men with TMSC < or ≥ 10 million showed that this association remained (p≤ 0.001) after adjustment for these confounders. In addition, after adjustment for confounders the sperm concentration was also positively associated with the use of sympathomimetics, but only in men with TMSC ≥ 10 million (β 0.300; 95% CI, 0.032-0.568).

Conclusions These first data show the potential benefits of the use of sympathomimetics to improve sperm motility in men of subfertile couples, which needs further investigation.

Key message

In this study, the use of sympathomimetics was positively associated with sperm motility in men of subfertile couples. Further investigation is necessary to test whether men of subfertile couples could benefit from this potential second use of sympathomimetics.

Introduction

Worldwide one out of six couples experiences subfertility, which is defined as the failure to conceive after 1 year of regular, unprotected intercourse with the same partner (199, 200). Of all subfertile couples, 20-30% is explained by male subfertility only and in 25-40% both male and female subfertility are found (200). Unfortunately, only a few medical treatments are available for male subfertility.

Research from the past two decades has shown that semen quality is influenced by several conditions and modifiable factors, such as obesity, nutrition, smoking and medication use (201-205). In the Netherlands, the overall use of medication of the male population of reproductive age (20 – 60 year) is approaching 55% (201). Subfertility resulting from medication-induced endocrine malfunction or injury of spermatogenesis is a largely neglected clinical problem (202-205). The exact potentially toxic effect on the gonads of many commonly used medicines is; however, still largely unknown (206-210), as well as the impact of chronic diseases on male fertility (202, 211, 212).

An example of a very common chronic disease with an overall prevalence in the Netherlands of 6% is asthma / bronchitis (213). According to the guidelines of the Dutch College of General Practitioners these chronic diseases are treated with short-acting inhaled sympathomimetics, a low/high dose inhaled glucocorticoids or combination of these medicines (214). In the Netherlands, around 5.6% of males of reproductive age uses medication for asthma / bronchitis (201). As the adrenergic receptors on which these medications act are also present in the reproductive system, we hypothesize that sympathomimetics can also affect spermatogenesis. Therefore, this study aims to assess associations between the use of sympathomimetics and sperm quality in men of subfertile couples.

Materials and methods

Study population

Between 2007 and 2012, couples contemplating pregnancy and visiting the outpatient clinic of the Department of Obstetrics and Gynaecology at the Erasmus MC, University Medical Centre Rotterdam, the Netherlands, were offered preconception counselling. This special outpatient clinic 'Achieving a healthy pregnancy' (39) was visited by 2,365

men of whom 2,166 provided written informed consent (METC Erasmus MC 2004-277). We excluded men of whom a semen analysis was not performed within 0 - 70 days prior to the visit or 21 days after the visit. This window covers the 10 weeks of spermatogenesis during which medication use was assessed and reduces confounding by counselling as this window was independent of exposures and bias due to the exclusion of conditions affecting semen parameters. We further excluded men with semen samples provided by microsurgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA) or retrograde ejaculation, and with incomplete data or no data on medication use. This resulted in 882 men for further evaluation (***Figure 1***). The study protocol has been approved by the Medical Ethical and Institutional Review Board of the Erasmus MC, University Medical Centre Rotterdam, the Netherlands on 25th June 2013.

Data collection

At study entry, all participants completed a self-administered questionnaire covering (non)medical conditions at enrolment which were thoroughly checked by the researcher during the preconception visit during which also anthropometric measurements (height, weight, BMI, waist-hip ratio, and blood pressure) were obtained (39).

Medication use

Information on medication use was obtained from self-administered questionnaires and was divided according the Anatomical Therapeutic Chemical (ATC) Classification System (215, 216). In our study population Short-acting β2 adrenergic receptor agonists (SABA) and Long-acting β2 adrenergic receptor agonists (LABA) were used for asthma / bronchitis, as well as inhaled corticosteroids (either alone or combined). Information on dosage and duration was not available.

Semen analysis

Semen samples were collected via masturbation after a required abstinence period of 3-5 days. Within one hour, samples were liquefied and the semen parameters ejaculate volume, sperm concentration, percentage progressive (type A+B) and immotile spermatozoa (type C+D) were assessed according to World Health Organization (WHO) guidelines (World health organization, 2010). Total sperm count was calculated as the product of ejaculate volume and sperm concentration. Total motile sperm count

(TMSC) was calculated as the product of ejaculate volume, sperm concentration, and percentage progressive motile sperm (type A+B). Normospermia was defined as TMSC ≥ 10 million. All semen analyses were performed by expert laboratory staff at the Erasmus MC, University Medical Centre Rotterdam, the Netherlands. Semen samples were not routinely collected as part of the study, but only on the clinical indication of subfertility. Semen parameters were therefore retrieved from medical records to obtain all the data required for this study.

Statistical analyses

Primary analyses included descriptive statistics providing characteristics of the study sample, expressed as medians with interquartile ranges or absolute numbers with percentages and were compared using either Mann-Whitney U or chi-squared tests. A normal distribution of the semen parameters was achieved with root transformation of ejaculate volume and sperm count, and fourth root transformation of sperm concentration and TMSC. Univariable and multivariable linear regression models were applied to study associations between the use of sympathomimetics and semen parameters.

After the crude analysis, we constructed an adjusted model including the potential confounders body mass index (BMI), alcohol use, smoking, geographic background, age, and folic acid supplement use based on their associations with semen parameters. Additionally, we adjusted for the four astronomical seasons. As a final step confounding by indication was addressed as this is a bias frequently encountered in observational epidemiologic studies of medication effects. To do so, we additionally adjusted for the presence or absence of asthmatic disease. Finally, we performed a similar multivariable linear regression analysis stratified for men with a TMSC < or ≥ 10 million. Estimates are expressed by regression coefficients (β) with confidence intervals (95% CI). All analyses were performed using SPSS for Windows (version 21.0, IBM SPSS,). P-values < .05 were considered statistically significant.

Results

General characteristics of the study population (n=882) and excluded population (n=1,483) are presented in ***Supplemental Table 1***. Included men were slightly younger

(34 vs 35 years; p<.001) and more often used alcohol (71.5 vs 66.9%; p<.05). Overall, the study population were aged 34 ± 4 years (mean ± SD), with a Body Mass Index (BMI) of 26.1 ± 2.3 kg/m^2 (mean ± SD), and sympathomimetic use was reported by 3.6%. ***Table 1*** shows the general characteristics of the study population divided in men using sympathomimetics (n=32) and those not using any medication (n=850). Sympathomimetic users showed a higher percentage of folic acid supplement use (24.1% vs 6.0%; p<.001) and a higher percentage progressive motile sperm (48% vs 38%; p<.05) than non-medication users (***Table 1***).

After adjustment for potential confounders, i.e. smoking, alcohol use, age, geographic background, BMI, folic acid supplement use and the four astronomical seasons, multivariable linear regression analyses revealed that the use of sympathomimetics compared with no medication use remained positively associated with a 10% higher percentage of progressive motile sperm (model 1; β = 10.859; 95% CI, 3.977-17.742). By additionally adjusting for asthmatic disease, the significantly positive association between the use of sympathomimetics and percentage progressive motile sperm remained (model 2; β = 10.265; 95% CI, 3.258-17.272) (***Table 2***).

In a subgroup analysis of men with TMSC < or ≥ 10 million, these positive associations remained, but only in men with TMSC < 10 million (***Supplemental Table 2***). Moreover, after adjustment for potential confounders, sperm concentration was also positively associated with the use of sympathomimetics, but was only statistically significant in men with TMSC ≥ 10 million (***Supplemental Table 2***). Stratifying the study population in men with TMSC < or ≥ 3 million and in men with TMSC < or ≥ 1 million did not significantly change the associations, although the use of sympathomimetics was only significantly associated with sperm motility in men with TMSC ≥ 1 and ≥ 3 million (model 2; β = 6.890; 95% CI, 1.409-12.371 and model 2; β = 8.205; 95% CI, 2.290-14.120 for TMSC ≥ 3 million and ≥ 1 million, respectively) (***Supplemental Table 3***). Moreover, in men with TMSC ≥ 3 million use of sympathomimetics was significantly associated with less ejaculate volume after adjustment for potential confounders (model 2; β = -0.196; 95% CI, -0.379- -0.012) (***Supplemental Table 3***).

As corticosteroids were also used by some men in the study population, associations of corticosteroid use with semen quality were also studied but showed no significant associations (data not shown).

Discussion

This study demonstrates that the use of sympathomimetics is associated with a significantly higher percentage progressive motile sperm (type A+B) independent of corticosteroid use. Moreover, in men with TMSC ≥ 10 million sperm concentration was also positively associated with the use of sympathomimetics. These positive associations remained after adjustment for potential confounders and confounding by indication.

Sympathomimetics are agonists for β-adrenergic receptors, a class of G protein-coupled receptors, resulting in stimulation of the sympathetic nervous system. This activation of β-adrenergic receptors is followed by activation of the enzyme adenylate cyclase which in turn leads to activation of the secondary messenger cyclic adenosine monophosphate (cAMP), which activates Protein Kinase A (PKA) (217). Testicular spermatozoa develop the ability for motility during the transit through the epididymis (218). Therefore, a possible explanation for the positive association between the use of sympathomimetics and percentage progressive motile sperm may be related to the increase in cAMP and PKA as these signalling pathways, together with the calcium pathway, are known to be the most central to the regulation of sperm motility (219). This hypothesis is substantiated by Esposito et al, showing a time-dependent recovery of sperm motility in mice with impaired motility, after loading with cAMP (220).

Several studies have indicated that normal function of the β-adrenergic receptor is necessary for normal contraction of the vas deferens and seminal vesicles and consequent sperm ejaculation (221, 222). By stimulation of the β-receptors, the vasoconstrictive effect of the β-receptors decreases. This may explain the negative trend we found between the use of sympathomimetics and ejaculate volume. The significantly positive association with sperm concentration found in the subgroup of men with TMSC ≥ 10 million is suggested to be a direct consequence of the decrease in ejaculate volume as sperm concentration is the product of sperm count multiplied by ejaculate volume.

The FDA states that sympathomimetics are safe to use on a daily basis for the indication of asthmatic disease, but surprisingly data on the effects on semen parameters are missing (223). In future, the use of sympathomimetics could be considered to enhance sperm motility in subfertile men, especially because of the relatively mild adverse effects and friendly route of administration. This is of interest because of the few therapeutic alternatives to enhance male fertility with selective oestrogen receptor modulators, such as Clomiphene citrate and Tamoxifen, with more frequent adverse effects than sympathomimetics, such as nausea, headache, alteration in libido, visual field changes and gynecomastia (224-226).

Our study is limited by the fact that only one semen analysis was taken into account which does not account for intra-individual variation of semen parameters. Since we have only included men as cases when they used the specific medication at the time of the actual study visit, the number of cases is relatively small. Some heterogeneity caused by an unknown combination of diseases and the fact that not every disease is always treated could have underestimated the estimates. Moreover, dose response effects could not be studied to further estimate causality of the observed associations. Lastly, this study was performed in men of subfertile couples and therefore the external validity is limited. However, the majority of men had normospermia with most parameters above the lower reference limit, which in contrast, does makes the results generalizable to all men.

Major strengths of our study are the standardised data collection by a trained researcher of a large group of men of subfertile couples visiting one tertiary hospital and the fact that semen analyses were performed at one laboratory. Moreover, the time period between measurement of sperm parameters and study entry visit was strictly set, so it reflects the sensitive exposure window of male spermatogenesis of approximately 70 days. Besides, several confounders, including the use of other medicines, were included in the analyses, confounding by indication was excluded and stratified analyses were performed in order to contribute to personalised medicine in the future.

Conclusion

Our study demonstrates that the use of sympathomimetics is positively associated with sperm motility in men of subfertile couples. Moreover, in men with TMSC ≥ 10 million use of sympathomimetics was also positively associated with sperm concentration. This explorative data suggests that male subfertility could be a new indication for second use of sympathomimetics, a novel finding which needs to be interpreted with caution and emphasize the need for further research on causality.

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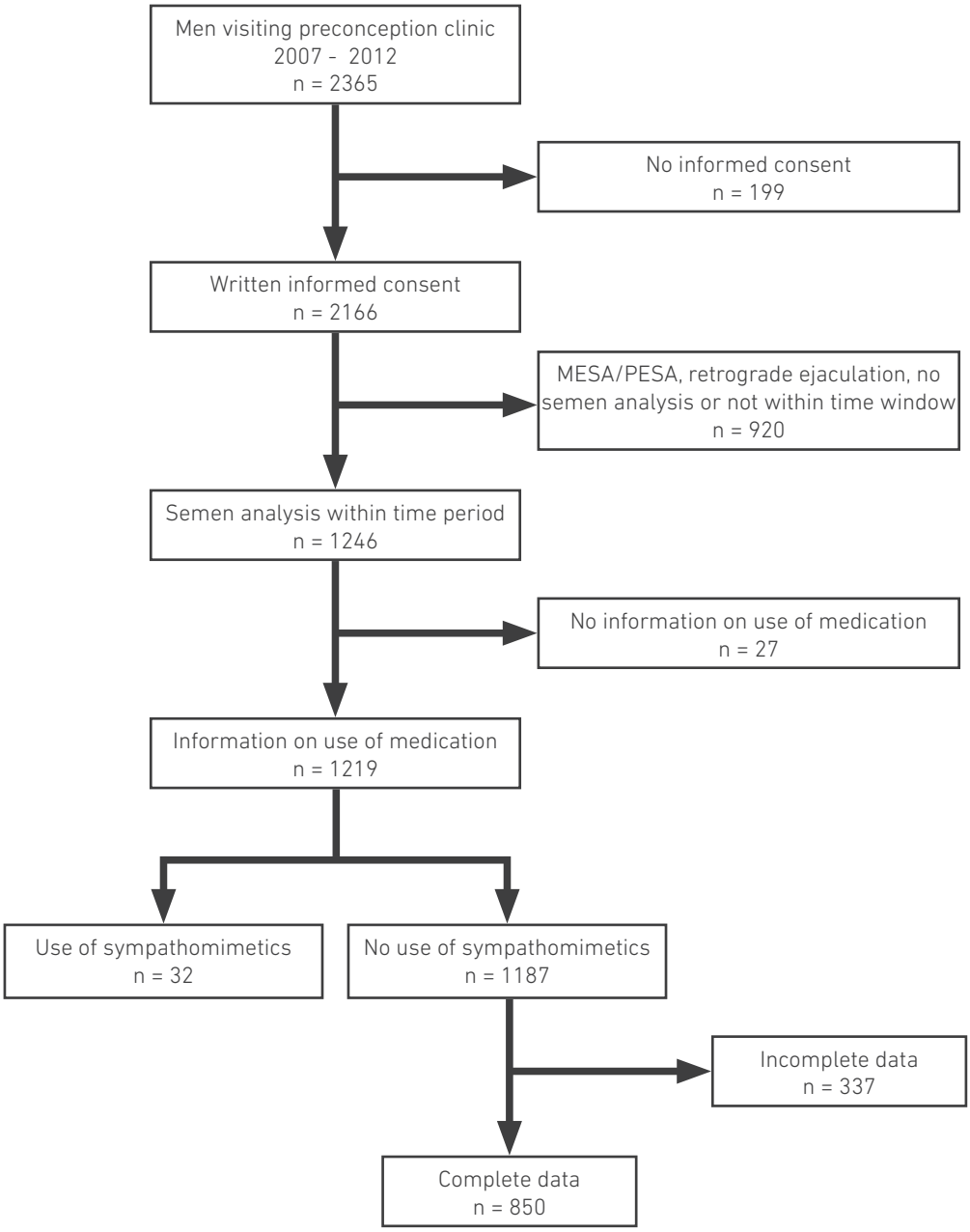
Conflict of interest

None of the authors have any conflict of interest related to the discussed topic.

Figure 1. Flow diagram of included and excluded men



MESA = microsurgical epididymal sperm aspiration; PESA = percutaneous epididymal sperm aspiration

Table 1. General characteristics of the study population of men of subfertile couples visiting the preconception outpatient clinic 'Achieving a healthy pregnancy' (n=882).

	Users of sympathomimetics (n=32)	**Non-medication users (n=850)**	
Age (years)	35 (32-39)	34 (30-39)[a]	
BMI (kg/m2)	27.4 (24.4-30.7)[b]	26.1 (23.9-28.5)[c]	
Geographic background (Dutch)	21 (65.6%)	538 (63.6%)[d]	
Smoking (Yes)	7 (21.9%)	281 (33.1%)[e]	
Alcohol (Yes)	27 (84.4%)	602 (71.0%)[f]	
Recreational drugs (Yes)	1 (3.1%)	65 (7.6%)	
Folic acid supplement use (Yes)	7 (24.1%)[g] **	45 (6.0%)[h]	
Semen parameters:			Lower reference limit (WHO 2010)
Ejaculate volume (ml)	2.75 (1.45-3.60)	2.80 (1.80-3.90)	1.5
Sperm concentration (10^6/ml)	33 (11-81)	26 (9-59)	15
Total sperm count (10^6/ejaculate)	60.8 (17.5-182)	64.8 (19.7-146)	39
Total motile sperm count (10^6/ejaculate)	30.8 (7.5-89.4)	24.5 (4.1-68.4)	10
Progressive motile sperm (A + B) (%)	48 (39-53)*	38 (23-49)	32
Duration of sexual abstinence (days)	4 (3-5)[i]	4 (3-7)[j]	

Values are expressed as median (IQR) or number (%); chi-squared and Mann Whitney Tests were performed: * p <.05 ** = p <.001; Missing items: two[a] for age, one[b] and 36[c] for BMI, four[d] for geographic background, one[e] for smoking, two[f] for alcohol, and three[g] and 45[h] for FA supplement use, 22[i] and 340[j] for duration of sexual abstinence. BMI = Body Mass Index;

Table 2. Uni- and multivariable linear regression models of men of subfertile couples (n=882).

Sympathicomimetics	**Ejaculate volume √(mL)** β (95% CI)	**Sperm concentration 4√(106/mL)** β (95% CI)	**Sperm count √(106/ejaculate)** β (95% CI)	**TMSC 4√(106/ejaculate)** β (95% CI)	**Sperm motility (A+B%)** β (95% CI)
Unadjusted (n=882)	-0.103 (-0.268 ; 0.063)	0.166 (-0.149 ; 0.481)	0.465 (-1.732 ; 2.662)	0.161 (-0.212 ; 0.534)	8.224 (1.798 ; 14.651)*
Model 1 (n=795)	-0.119 (-0.298 ; 0.060)	0.287 (-0.049 ; 0.623)	1.123 (-1.210 ; 3.456)	0.301 (-0.097 ; 0.699)	10.859 (3.977 ; 17.742)*
Model 2 (n=795)	-0.111 (-0.293 ; 0.072)	0.279 (-0.062 ; 0.621)	1.093 (-1.284 ; 3.470)	0.285 (-0.121 ; 0.690)	10.265 (3.258 ; 17.272)*

Note: data depicted as β and confidence interval (95% CI) * = p <0.05. The regression coefficient (β) indicates the increase or decrease (-) change per unit of the sperm parameter.
Model 1: adjusted for smoking, alcohol use, age, geographic background, BMI, folic acid supplement use and the four astronomical seasons
Model 2: model 1 with additional adjustment for asthma / bronchitis

Supplemental Table 1. General characteristics of the study population and the excluded population (n=2365).

	Study population (n=882)	Excluded population (n=1483)
Age (years)	34 (30-39)[a]**	35 (31-40)[b]
BMI (kg/m2)	26.1 (23.9-28.6)[c]	26.3 (23.9-29.1)[d]
Geographic background (Dutch)	559 (63.7%)[e]	801 (59.6%)[f]
Smoking (Yes)	288 (32.7%)[g]	428 (31.7%)[h]
Alcohol (Yes)	629 (71.5%)[i]*	905 (66.9%)[j]
Recreational drugs (Yes)	66 (7.5%)	112 (8.3%)[k]
Folic acid supplement use (Yes)	52 (6.2%)[l]	93 (7.2%)[m]
Use of sympathomimetics (Yes)	32 (3.6%)**	23 (1.7%)[n]
Asthma / Bronchitis (Yes)	47 (5.3%)	49 (3.3%)

Values are expressed as median (IQR) or number (%); chi-squared and Mann Whitney Tests were performed: * = p <0.05, ** = p <.001; Missing items; two[a] and 140[b] for age, 37[c] and 220[d] for BMI, four[e] and 140[f] for geographic background, one[g] and 131[h] for smoking, twoi and 130[j] for alcohol, 129[k] for recreational drugs, 48[l] and 193[m] for FA supplement use, and 150[n] for use of sympathomimetics. BMI = Body Mass Index;

Supplemental Table 2. Uni- and multivariable linear regression models in men of subfertile couples, stratified by TMSC < or ≥ 10 million.

Sympathicomimetics		**Ejaculate volume √(mL)** β (95% CI)	**Sperm concentration 4√(106/mL)** β (95% CI)	**Sperm count √(106/ejaculate)** β (95% CI)	**TMSC 4√(106/ejaculate)** β (95% CI)	**Sperm motility (A+B%)** β (95% CI)
Unadjusted (n=882)	TMSC <10	-0.061 (-0.360 ; 0.238)	0.058 (-0.368 ; 0.485)	-0.269 (-1.631 ; 1.094)	0.148 (-0.203 ; 0.499)	13.696 (4.227 ; 23.165)*
	TMSC ≥10	-0.128 (-0.322 ; 0.067)	0.203 (-0.045 ; 0.450)	0.707 (-1.631 ; 3.045)	0.135 (-0.149 ; 0.420)	5.078 (-0.028 ; 10.184)
Model 1 (n=795)	TMSC <10	-0.020 (-0.355 ; 0.314)	0.146 (-0.334 ; 0.625)	0.145 (-1.367 ; 1.656)	0.362 (-0.036 ; 0.759)	20.709 (10.029 ; 31.389)**
	TMSC ≥10	-0.166 (-0.374 ; 0.042)	0.290 (0.027 ; 0.554)*	1.210 (-1.254 ; 3.674)	0.189 (-0.110 ; 0.489)	5.691 (0.374 ; 11.007)*
Model 2 (n=795)	TMSC <10	-0.011 (-0.352 ; 0.329)	0.132 (-0.357 ; 0.620)	0.153 (-1.386 ; 1.693)	0.336 (-0.068 ; 0.741)	19.760 (8.900 ; 30.621)**
	TMSC ≥10	-0.157 (-0.368 ; 0.055)	0.300 (0.032 ; 0.568)*	1.250 (-1.259 ; 3.758)	0.197 (-0.108 ; 0.501)	5.427 (0.015 ; 10.839)*

Note: data depicted as β and confidence interval (95% CI) * = p <0.05, ** = p<0.001 The regression coefficient (β) indicates the increase or decrease (-) change per unit of the sperm parameter. a n = 313 for TMSC <10 and n = 569 for TMSC ≥10 in the unadjusted model; b n = 277 for TMSC <10 and n = 518 for TMSC ≥10 in adjusted model 1 and 2.
Model 1: adjusted for smoking, alcohol use, age, geographic background, BMI, folic acid supplement use and the four astronomical seasons
Model 2: model 1 with additional adjustment for asthma / bronchitis

Supplemental Table 3. Uni- and multivariable linear regression analyses in men of subfertile couples, stratified by TMSC < or ≥ 3 million, and < or ≥ 1 million.

Sympathicomimetics		**Ejaculate volume √(mL)** β (95% CI)	**Sperm concentration 4√(106/mL)** β (95% CI)	**Sperm count √(106/ejaculate)** β (95% CI)	**TMSC 4√(106/ejaculate)** β (95% CI)	**Sperm motility (A+B%)** β (95% CI)
Unadjusted (n=882)	TMSC <3	0.255 (-0.241 ; 0.750)	-0.385 (-1.069 ; 0.299)	-1.026 (-2.788 ; 0.736)	-0.315 (-0.787 ; 0.156)	-4.984 (-18.673 ; 8.705)
	TMSC ≥3	-0.174 (-0.345 ; -0.002)*	0.072 (-0.163 ; 0.307)	-0.359 (-2.470 ; 1.752)	0.005 (-0.279 ; 0.289)	6.916 (1.729 ; 12.103)*
	TMSC <1	0.252 (-0.255 ; 0.759)	-0.118 (-0.752 ; 0.516)	-0.254 (-1.618 ; 1.111)	-0.082 (-0.467 ; 0.302)	-2.281 (-16.695 ; 12.132)
	TMSC ≥1	-0.159 (-0.332 ; 0.014)	0.148 (-0.098 ; 0.394)	0.247 (-1.911 ; 2.405)	0.120 (-0.186 ; 0.426)	8.830 (3.231 ; 14.428)*
Model 1 (n=795)	TMSC <3	0.641 (-0.081 ; 1.364)	-0.611 (-1.587 ; 0.365)	-1.287 (-3.840 ; 1.266)	-0.210 (-0.890 ; 0.470)	2.805 (-17.259 ; 22.868)
	TMSC ≥3	-0.206 (-0.387 ; -0.025)*	0.115 (-0.131 ; 0.361)	-0.139 (-2.341 ; 2.063)	0.014 (-0.281 ; 0.310)	7.074 (1.680 ; 12.468)*
	TMSC <1	0.580 (-0.174 ; 1.333)	-0.320 (-1.237 ; 0.597)	-0.560 (-2.544 ; 1.423)	-0.002 (-0.561 ; 0.557)	6.107 (-15.523 ; 27.738)
	TMSC ≥1	-0.189 (-0.371 ; -0.007)*	0.177 (-0.080 ; 0.434)	0.375 (-1.867 ; 2.616)	0.112 (-0.206 ; 0.430)	8.546 (2.725 ; 14.367)*
Model 2 (n=795)	TMSC <3	0.722 (-0.130 ; 1.573)	-0.778 (-1.927 ; 0.371)	-1.413 (-4.421 ; 1.596)	-0.188 (-0.990 ; 0.614)	8.902 (-14.681 ; 32.485)
	TMSC ≥3	-0.196 (-0.379 ; -0.012)*	0.134 (-0.116 ; 0.384)	-0.019 (-2.256 ; 2.218)	0.031 (-0.270 ; 0.332)	6.890 (1.409 ; 12.371)*
	TMSC <1	0.535 (-0.381 ; 1.451)	-0.625 (-1.735 ; 0.485)	-1.444 (-3.837 ; 0.949)	-0.151 (-0.828 ; 0.527)	11.511(-14.740 ; 37.762)
	TMSC ≥1	-0.178 (-0.363 ; 0.007)	0.190 (-0.071 ; 0.451)	0.447 (-1.831 ; 2.725)	0.119 (-0.204 ; 0.442)	8.205 (2.290 ; 14.120)*

Note: data depicted as β and confidence interval (95% CI) * = p <0.05. The regression coefficient (β) indicates the increase or decrease (-) change per unit of the sperm parameter. a n = 198 for TMSC <3, n = 684 for TMSC ≥3, n = 138 for TMSC <1 and n =744 for TMSC ≥1 in the unadjusted model; b n = 172 for TMSC <3, n = 623 for TMSC ≥3, n = 121 for TMSC <1 and n = 674 for TMSC ≥1 in adjusted model 1 and 2.
Model 1: adjusted for smoking, alcohol use, age, geographic background, BMI, folic acid supplement use and the four astronomical seasons
Model 2: model 1 with additional adjustment for asthma / bronchitis

The use of the mHealth program Smarter Pregnancy in preconception care

rationale, study design and data collection of a randomized controlled trial

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Matthijs R. van Dijk
Elsje C. Oostingh
Maria P.H. Koster
Sten P. Willemsen
Joop S.E. Laven
Régine P.M. Steegers-Theunissen

Abstract

Background

Unhealthy nutrition and lifestyle contribute to the worldwide rising prevalence of non-communicable diseases. This also accounts for the reproductive population, in which unhealthy behavior affects fertility and pregnancy outcome. Maternal smoking, alcohol consumption and inadequate folic acid supplement use are strongly associated with fetal complications as small for gestational age, premature birth and congenital malformations. In the Netherlands 83% of the perinatal mortality rate is due to these complications and is relatively high compared to other European countries. In order to reduce this prevalence rate, preconception care should be focused on the promotion of health of prospective parents by identification and intervention on modifiable nutrition and lifestyle risk factors. We developed the personal mHealth program "Smarter Pregnancy" (Dutch version available on: www.slimmerzwanger.nl) to provide individual coaching and information to improve nutrition and lifestyle during the preconception period in order to improve health of the reproductive population and subsequent generations.

Methods

Women between 18 and 45 years of age, and trying to conceive are eligible for inclusion in a randomized controlled trial. Participants are allocated either to a general population cohort or a subfertile (IVF/ICSI) population cohort. The intervention group receives personal online coaching based on the identified nutrition and lifestyle risk factors at baseline. Coaching comprises recipes, incentives, additional questions including feedback and text and e-mail messages, with a maximum of three per week. The control group only receives one recipe per week to maintain adherence to the program and prevent drop out. Screening questionnaires are send in both groups at 6, 12, 18, and 24 weeks of the program to monitor the change in the identified risk factors.

Discussion

We expect to demonstrate that the mHealth program Smarter Pregnancy can effectively improve nutrition and lifestyle in couples contemplating pregnancy. By the identification and improvement of modifiable nutrition and lifestyle risk factors on a large scale, both reproductive and pregnancy outcomes can be improved and subsequent perinatal

morbidity and mortality rates are expected to be reduced. The current use and rapid development of mHealth applications offers new opportunities to reach and educate large populations, which can facilitate the implementation of preconception care.
Trial registration: Dutch trial register: NTR4150 (available on: www.trialregister.nl).

Introduction

Unhealthy nutrition and lifestyle, characterized by a high caloric intake and vitamin deficiencies, derange metabolic and endocrine pathways and are causing obesity which contributes to the development of non-communicable diseases (NCDs), such as cardiovascular and metabolic diseases (227, 228). Although awareness of the impact of unhealthy nutrition and lifestyle is increasing, its prevalence remains very high, not only in general, but also in the reproductive population in which health consequences range from subfertility to congenital malformations or even perinatal death (25, 39, 106, 229, 230). Most evidence is available on the detrimental impact of maternal smoking, alcohol consumption and inadequate folic acid supplement use, which are strongly associated with embryonic growth and small for gestational age (SGA) and congenital malformations (50, 231-234). Currently, several studies that focused on the adherence of maternal dietary patterns have shown the benefits of healthy foods such as fruits and vegetables on perinatal outcome (235, 236).

In the Netherlands, particularly in large cities such as Rotterdam, perinatal mortality rates and the prevalence of perinatal complications, such as SGA, premature birth and congenital malformations (also referred to as BIG3 complications), is relatively high compared to other European countries (237-239). In order to reduce these prevalence rates, preconception care (PCC) should be implemented, focused on the promotion of health and the identification of (modifiable) risk factors of prospective parents as well as the next generation(5, 240, 241).

In order to create awareness and to implement PCC on a large scale, new approaches need to be explored and (mobile) technologies can be used. Previously, we developed and implemented a preconception outpatient clinic tailored to improve nutrition and lifestyle of which the results were promising, i.e. 30% reduction of inadequate nutrition and lifestyle and a 65% increased chance of ongoing pregnancy after IVF treatment (39, 242). However, this outpatient clinic could only provide PCC on a small scale due to the required expertise, time and costs. To overcome these barriers we have developed the personal mHealth coaching program Smarter Pregnancy (Dutch version available on: www.slimmerzwanger.nl, English equivalent available on: www.smarterpregnancy.co.uk/research), providing individual, tailored and continuous information on a large scale

during 26 weeks. Previous studies have shown that women seek online information with regard to healthy nutrition and lifestyle which suggests that online interventions using mobile technology can be effective (24, 243). Also, women embrace online anonymity to control and self-manage online information (244, 245). Smarter Pregnancy identifies the most important risk factors regarding nutrition and lifestyle and subsequently provides tailored information and motivational coaching by text and e-mail messages (25).

We hypothesize that our mHealth program will effectively improve nutrition and lifestyle in couples contemplating pregnancy. Based on our previous studies and that of others we designed a randomized controlled trial (RCT) to study the effectiveness of Smarter Pregnancy, defined as a significant improvement of vegetable and fruit intake and folic acid supplement use, when started preconceptional (25, 39, 230, 242, 246). This intervention can be considered as a primary prevention tool resulting in a reduction of Big-3 complications, perinatal morbidity and mortality in the short-term and NCDs in the long-term (228, 247, 248).

Objectives

A randomized controlled trial is conducted in two independent populations, i.e. couples from the general population and couples undergoing IVF/ICSI treatment, to study whether unhealthy nutrition and lifestyle can be improved by the Smarter Pregnancy coaching program as an intervention tool. Furthermore, we will determine whether couples will have a higher pregnancy rate and if their risk for BIG3 complications can be reduced by improving nutrition and lifestyle.

Primary outcome:

Improvement (percentage reduction) of unhealthy nutrition and lifestyle in women and men contemplating pregnancy or already pregnant, determined by using a dietary risk score (DRS), 24 weeks after starting the Smarter Pregnancy intervention.

Secondary outcomes:

1) A reduction in smoking by women and men contemplating pregnancy; 2) pregnancy rates in couples; 3) birth prevalence rate of BIG3 complications in the entire study population; 4) cost-effectiveness of the Smarter Pregnancy intervention.

Tertiary outcomes:

The influence of participation of men, pregnancy, age, low socioeconomic status on the primary outcome and 1) Improvement (defined as the percentage of reduction) of unhealthy nutrition and lifestyle 36 weeks after starting the Smarter Pregnancy intervention; 2) the compliance and reliability of Smarter Pregnancy among both women and men. To study the latter, we aim to determine the: 1) The percentage of the target group that meets all the inclusion criteria for the study, but does not participate; 2) The percentage of participants that is still participating after three months (compliance); 3) The prevalence and nature of technical problems.

Study design

Eligibility

Women residing in the Netherlands who are between 18 and 45 years of age and contemplating pregnancy are considered eligible to be included in this multi-center study. To participate, women need to be in possession of a smartphone with Internet access. Women with insufficient knowledge or understanding of the Dutch language, women who are treated by a dietician to lose weight in the context of a fertility treatment, and women who have a specific diet (e.g. vegans) cannot participate in the study. Male partners are also invited to participate, but only if they meet the same criteria, except that there is no upper age limit for male participants.

Recruitment, cohort composition and randomization

Women are invited to participate by a (health care) professional from their midwifery practice, children's daycare, childhealth center, or hospital. Self-registration through the website is also possible. Potentially eligible participants are contacted after registration by one of the researchers to verify their eligibility, to provide more details and answer questions about Smarter Pregnancy and to confirm their registration.

Participants are allocated either in a general population cohort or the IVF/ICSI-(ART) population cohort, depending on whether they will receive fertility treatment. Randomization of the participants is stratified by cohort and per center of inclusion. For each stratum a permuted block design is used and programmed beforehand. Hereby, allocation concealment is ensured.

Smarter Pregnancy

The mHealth program Smarter Pregnancy was launched in 2012 and provides personal coaching, tailored on personal conditions, gender, nutrition and lifestyle in both women and men contemplating pregnancy. The program is based on nearly 30 years of research and expertise by our group on the influence of nutrition and lifestyle on reproduction and pregnancy course and outcome. We used elements of Prochaska and Diclemente's transtheoretical model with a focus on the readiness for behavioral change, Bandura's social cognitive theory for self-efficacy and Fogg's behavior model to include triggers to motivate and increase the ability to change (249-251). Features of the attitude, social influence, and self-efficacy (ASE) model for coaching are applied; aimed at the understanding and motives of people to engage in specific behavior (252).

Intervention group

The content of the individual coaching is based on the baseline screening on personal conditions, nutrition and lifestyle and monitoring questionnaires at 6, 12, 18, and 24 weeks of the Smarter Pregnancy program. At these time points, participants are invited to complete a short, online questionnaire to monitor the change in their nutrition and lifestyle. Results from the questionnaires are compared with the previous results and shown on a personal online page to show a participant's progress.

The tailored coaching includes a maximum of three interventions per week comprising short message service (SMS) text and email messages containing tips, recommendations, vouchers, seasonal recipes, and additional questions addressing behavior, pregnancy status, body mass index (BMI) or adequacy of the diet (***Figure 1***, arrows pointing downwards).

The personal page also provides access to additional modules (i.e. applications) to support physical activity, an agenda to improve the compliance with hospital appointments and medicine adherence, and a module to monitor the safety of prescribed medication. A summary of all individual results can be obtained at any moment by the participant, and can be handed over or sent by email to the health care professional for further evaluation and support of preconception and antenatal care.

Control group

Participants who are randomized in the control group will not receive personal coaching after the baseline screening. They do receive access to their personal page and will receive one seasonal recipe per week to maintain adherence and prevent drop out (***Figure 1***, arrows poiting upwards). At baseline as well as at 12 and 24 weeks, participants in the control group receive the monitoring questionnaire about nutrition and lifestyle, but without feedback on the results. Also, every 6 weeks the controls receive a request to adjust their pregnancy status if needed.

Biomarker validation

To validate the self-administered questionnaires, we will analyze several blood biomarkers in a random sample of both study populations and both groups (intervention and control group). A team of qualified medical students will take blood samples at the participants home address or at the hospital. These blood samples will be taken on three time points (t = 0, 12 and 24 weeks) during the study; each time 20 ml will be collected. Samples are kept at -20 degrees Celsius for a maximum time period of 4 hours. Aliquots of residual blood will be stored at -80 degrees Celsius for future research on DNA and epigenetics.

Additional study questionnaires and follow-up

At baseline, for both the intervention and the control group additional information on social and demographic characteristics is obtained using an additional online study questionnaire implemented in the coaching program. The first follow-up study questionnaire will be send at 36 weeks, i.e. 12 weeks after the last screening moment (***Figure 2***). One year after enrollment, participants receive their last study questionnaire, which consists of questions regarding medical and obstetric history, medication use, whether they became pregnant during enrollment and, if applicable, the pregnancy outcome.

Ethics, dissemination and data

This trial will be conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients have been approved by the Medical Ethical and Institutional Review Board of the Erasmus MC, University Medical Centre, Rotterdam,

in the Netherlands (trial protocol version 6.0 d.d. 8th July 2013, NL40414.078.12). Digital and written informed consent (model consent form and patient information form (PIF) are added to this manuscript as an appendix) will be obtained from all participants to use the data for analyses. All data will be anonymously processed. Participants will be able to resign at any time, without any statement of reasons. During the trial, no concomitant care or interventions are prohibited. The investigator may also decide to remove a participant from the study in case of (emergent) medical reasons, but we consider this highly unlikely.

This mHealth program complies with the highest rules of legislation for medical devices in Europe; therefore, it has received the Conformité Européenne, classe 1 (CE-1), classification (2013) and can be used to improve the quality of medical care.

Due to the nature of the intervention, we consider the risk of (serious) adverse events non existing. Therefore, a data monitoring committee was not needed. We inform the funding organizations yearly about the progress of the trial. Currently, we have no plans regarding important protocol modifications, but we will communicate any modifications to all people involved.

The final analyzed dataset of this trial will be available to all involved researchers and collaborators. Our data will be available upon request from the corresponding author.

Statistical considerations

Sample size calculations are based on our primary outcome measure (DRS). Based on our previous studies and the survey using Smarter Pregnancy, we expect a reduction of approximately 0.5 DRS points (based on a standard deviation of 2.7) in the intervention group compared to the control group. Considering alpha=0.05 and power=0.80 we will need to include a total of 916 women in our study (2 arms of 458 each). Due to expected drop outs of approximately 10%, we aim to include 1,000 fertile (2 arms of 500 each) and 1,000 subfertile women (2 arms of 500 each) in our study. For 50% of these women, we expect their male partner (n=250 in each arm) to participate as well. Due to the lower SD (2.0) in men, with this sample size we are also able to demonstrate a reduction of at least a 0.5 DRS points in the male partners.

Statistical analysis

A flowchart will be used to depict the total participants of each cohort and divided per group, subdivided per gender. Also, the amount of resigning participants will be shown per time point (6 weeks). General and baseline characteristics will be compared between groups and shown in a baseline table.

The primary analysis will be based on intention to treat (ITT). For men and women in both the intervention and the control group the DRS will be calculated at baseline and after 24 weeks and used for further analyses. This continuous outcome measure will be analyzed by the 'difference in difference principle' and used in a linear regression model, including the initial/baseline value of the DRS. Repeated measurements will be used to investigate the effects of the intervention over time and the interaction of the intervention with socio economic status, ethnicity and age. Chi-square analysis and ANCOVA will be used to study the effects of the intervention on the pregnancy outcome and BIG3 complications.

To measure the compliance and reliability of Smarter Pregnancy we will analyze the percentage of randomized women who fill in the questionnaire after 12 weeks of participation and the percentage of participants who experienced technical problems. Corresponding confidence intervals will be given.

The influence on the primary outcome of participation of men, if pregnancy occurred during participation, age and low socio economic status will be analyzed by including these variables and their interaction with both groups, one by one in the model which will be used for the primary outcome. If there is heterogeneity of the treatment effect, the effect will be determined per subgroup separately.

Discussion

This study will contribute to the implementation of easily accessible PCC in order to increase awareness regarding the importance of healthy nutrition and lifestyle in couples contemplating pregnancy and health care professionals. Subsequently, this can reduce the relatively high rates of perinatal morbidity and mortality (BIG3 complications) in the Netherlands.

Initiating behavioral change(s) by the identification of risk factors during the preconception period can be a useful first step to not only create awareness, but also to lower the threshold to approach a healthcare professional during this period. Discussing or revealing involuntary childlessness remains a burden for many women as well as for men, due to the perception that they have failed by not being able to conceive. This results in a situation in which risk factors for poor reproductive and pregnancy outcome persist, while adopting a healthy lifestyle during this preconception period can be beneficial on both the short and long term. Most reproductive failures originate due to deranged metabolic pathways. The lack of co-factors and substrates as a result of vitamin deficiencies (e.g. vitamin B12 and folate) can influence oocyte en semen quality and early embryonic development resulting in failed implantation and miscarriages. Also, it can cause epigenetic modifications to DNA methylation of the offspring (5, 178, 253). Therefore, we consider the preconception period as the window of opportunity to initiate a healthy lifestyle.

Currently, research in the field of mobile technology is mainly aimed on the use of mHealth in low- and middle-income countries, because this new form of health care delivery can reach the poorest regions in which the prevalence of NCDs and poor maternal and child health are the highest (254-257). By our opinion, also high income countries comprise specific target groups, such as the reproductive population, in which risks for poor reproductive and pregnancy outcome accumulate, because of the lack of knowledge and self-efficacy with regard to PCC (258). Therefore, we consider mHealth a promising method to approach the large group of reproductive women and men which is currently wrongly assumed to be at low risk for poor reproductive and pregnancy outcome, although it is known that the prevalence of risk factors in this population is high (25, 39, 234). Given that 98.7% of all Dutch women and men between 18 and 45 years old have access to the internet and 95.4% can access the internet by their mobile phone makes this mHealth approach justifiable (259).

Strengths of this RCT are the longitudinal observations and the longitudinal biomarker validation in blood. Also, additional study questionnaires for short-term and long-term follow-up (respectively 12 and 26 weeks after the last questionnaire at 24 weeks), including sociodemographic data and medical record validation, are considered

important strengths of this study. A limitation of this RCT is the potential selection bias, which is unfortunately inherent to participation in a study, especially on behavioral change, as well as the exclusion of participants without sufficient knowledge of the Dutch language.

With this RCT we expect to demonstrate the effectiveness of our Smarter Pregnancy program and its positive effect on reproductive and pregnancy outcome in both fertile and subfertile couples. Healthcare professionals are often also not aware of the importance of PCC nor have tools containing information and guidelines to provide nutrition and lifestyle care for medical practice (260). Therefore, we consider this study a unique intervention regarding the implementation of accessible preconception care.

Funding

This research was funded by the Department of Obstetrics and Gynecology, Erasmus MC, University Medical Centre, Rotterdam, the Netherlands, a grant of ZonMW Health Care Efficiency Research and the Erasmus MC Mrace program 'Health Care Efficiency Research'.

Conflict of interest

From 2016 RST is CEO of eHealth Care Solutions and CSO of Slimmere Zorg BV. Other authors declare that they have no competing interests.

List of abbreviations

ART	Assisted reproductive therapy
ASE-model	Attitude, social influence and self-efficacy model
BMI	Body mass index
CE-1	Conformité Européenne, classe 1
DNA	Deoxyribonucleic acid
DRS	Dietary risk score
IVF	In vitro fertilization
ITT	Intention to treat
ICSI	Intracytoplasmic sperm injection
mHealth	Mobile health

NCD	Non-communicable disease
PCC	Preconception care
PIF	Patient information form
RCT	Randomized controlled trial
SD	Standard deviation
SGA	Small for gestational age
SMS	Short message service

Figure 1. Overview of the recruitment and composition of the multi-center study and both cohorts.

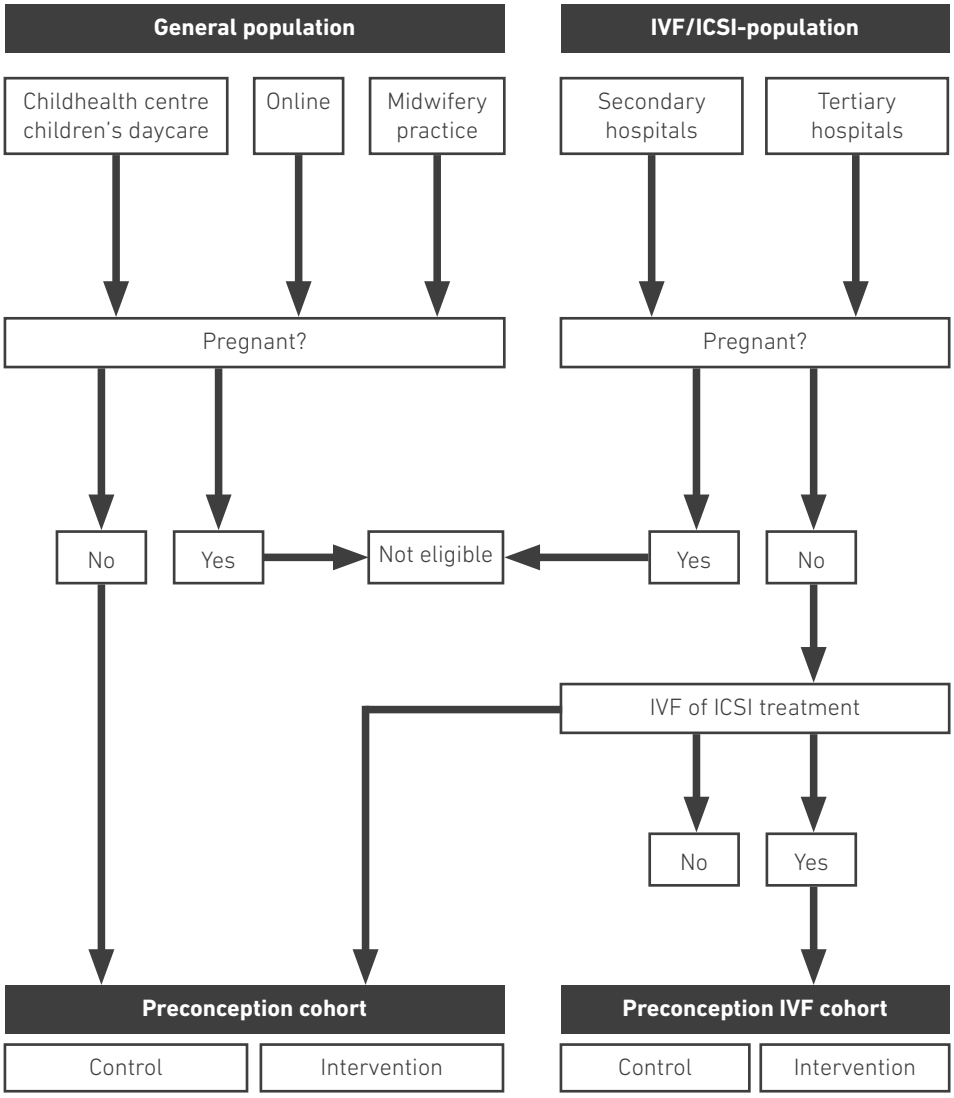
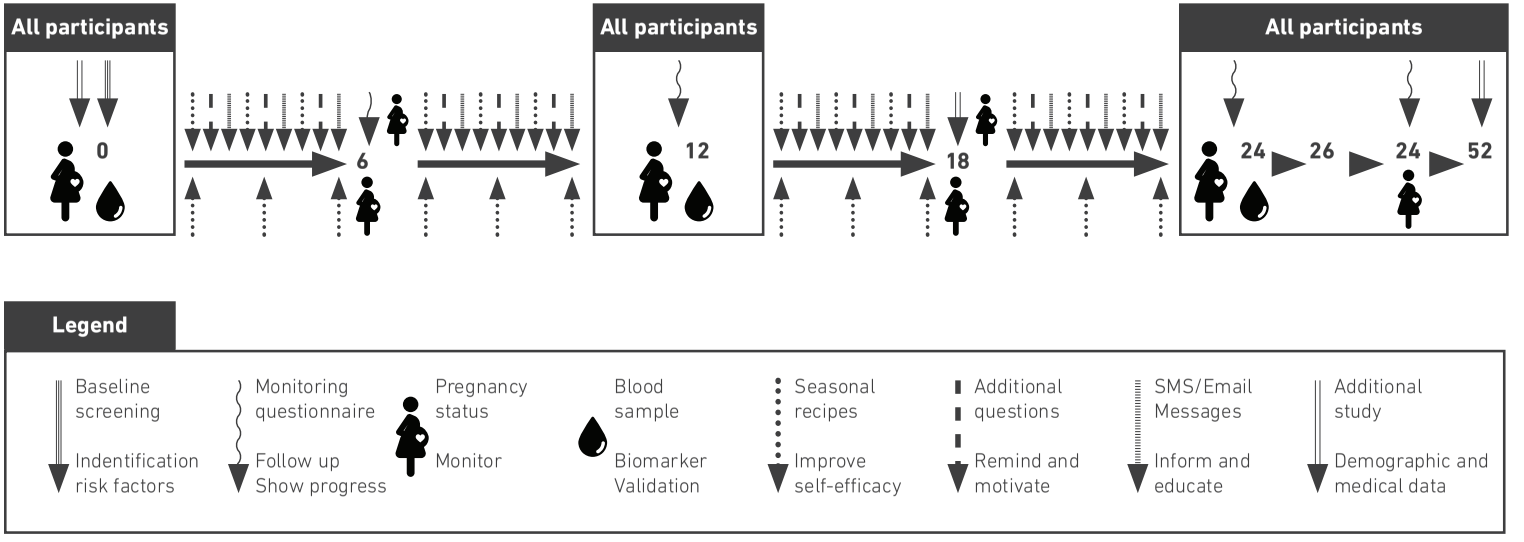


Figure 2. Overview of both the intervention and control group during their enrollment. The upper arrows pointing downwards depict the intervention group. The lower arrows pointing upwards depict the control group. All boxed icons depict aspects of the trial that account for all participants in both groups, i.e. baseline screening, screening questionnaires (at t=12 and t=24 weeks), additional questionnaires at baseline and 52 weeks, pregnancy status per 6 weeks and blood samples.



SPIRIT-Table

			Study period					
	Enrollment	**Allocation**		**Post-allocation**				**Close-out**
Timepoint (weeks)		t_0	t_6	t_{12}	t_{18}	t_{24}	t_{36}	t_{52}
Eligibility screen	X							
Informed consent	X							
Allocation		X						
INTERVENTION GROUP								
Screening questionnaire		X	X	X	X	X	X	
Additional questionnaire		X						X
Coaching			●	●	●	●		
Pregnancy status		X	X	X	X	X	X	
Blood collection*		X		X		X		
CONTROL GROUP								
Screening questionnaire		X		X		X	X	
Additional questionnaire		X						X
Coaching								
Pregnancy status		X	X	X	X	X	X	
Blood collection*		X		X		X		
ASSESSMENTS								
Baseline Age Length Weight BMI Vegetable intake Fruit intake Folic acid supplementation Smoking Alcohol consumption Pregnancy status Physical activity Demographics		X						
Outcome variables Weight BMI Vegetable intake Fruit intake Folic acid supplementation Smoking Alcohol consumption Pregnancy status Physical activity		X	X	X	X	X	X	
Blood collection Nutrients		X		X		X		
Follow-up Medical history Pregnancy outcome								X

* Determined in a random sample.
BMI, body mass index; RBC, red blood cell count; Hb, hematoglobin; Ht, hematocrite.

First effective mHealth nutrition and lifestyle coaching program for subfertile couples undergoing in vitro fertilization treatment

a single-blinded multicenter randomized controlled trial.

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Elsje C. Oostingh
Maria P.H. Koster
Matthijs R. van Dijk
Sten P. Willemsen
Frank J.M. Broekmans
Annemieke Hoek
Marriete Goddijn
Nicole F. Klijn
Evert J.P. van Santbrink
Eric A.P. Steegers
Joop S.E. Laven
Régine P.M. Steegers-Theunissen

Abstract

Objective To study compliance and effectiveness of the mHealth nutrition and lifestyle coaching program "Smarter Pregnancy" in couples undergoing in vitro fertilization (IVF) treatment with or without intracytoplasmic sperm injection (ICSI).

Design Multicenter, single blinded, randomized controlled trial, conducted between July 2014 and March 2017.

Setting Six IVF clinics throughout the Netherlands.

Patients 626 women undergoing IVF treatment with or without ICSI and 222 male partners.

Interventions Couples were randomly assigned to the light (control group) or regular (intervention group) Smarter Pregnancy program. Both groups filled out a baseline screening questionnaire on nutrition and lifestyle behaviors butthe intervention group received coaching tailored to inadequate behaviors during the 24-week period.

Main Outcome Measures Difference in improvement of a composite dietary and lifestyle risk score (DRS, LRS) for the intake of vegetables, fruits, folic acid supplements, smoking and alcohol use after 24 weeks of the program.

Results Compared with controls, women (β=0.779, 95%CI 0.456 to 1.090) and men (β=0.826, 95%CI 0.416 to 1.284) in the intervention group showed a significantly larger improvement of inadequate nutrition behaviors after 24 weeks of coaching. At the same time, these women also showed a significantly larger improvement of inadequate lifestyle behaviors (β=0.108, 95%CI 0.021 to 0.203).

Conclusion The mHealth coaching program Smarter Pregnancy is effective and improves the most important nutritional and lifestyle behaviors among couples undergoing IVF/ICSI treatment. International multicenter randomized trials are recommended to study the effect of using Smarter Pregnancy on pregnancy, live births, and neonatal outcome.

Trial registration number Dutch Trial Register: NTR4150,
http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4150

Capsule In subfertile couples undergoing IVF/ICSI treatment, the mHealth coaching program Smarter Pregnancy demonstrates high compliance and effectiveness to improve inadequate nutrition and lifestyle behaviors.

Introduction

Poor nutrition and lifestyle behaviors are still very common risk factors for many non-communicable diseases, including reproductive disorders, with an estimated 49 million couples coping with subfertility worldwide (1, 261). Nowadays assisted reproductive techniques, such as in vitro fertilization (IVF) treatment with or without intracytoplasmic sperm injection (ICSI), show highly acceptable cumulative ongoing pregnancy rates (fresh plus frozen) per initiated cycle (36.2% ongoing pregnancies in 2017 in the Netherlands, 33.4% ongoing pregnancies in 2016 in the United States (262, 263). However, these rates may be improved by adopting healthier nutritional and lifestyle behaviors (242). Unfortunately, most couples contemplating pregnancy, including subfertile couples at which a clinical pregnancy has not occurred after at least 12 months of regular unprotected intercourse, but also health care professionals, are usually not aware of the impact of nutritional and lifestyle behaviors on reproductive outcomes. Raising awareness by providing information and motivating these couples to change behaviors remains challenging (264).

As stated by Barker et al (265), there are four preconception action phases (i.e. children and adolescents, adults with no immediate intention to become pregnant, adults with intention to become pregnant, adults with intention to become pregnant again) in relation to the goal to become a parent, each with their own features and intervention strategies. A modern and potentially effective intervention strategy to initiate behavioral changes is the mobile phone with internet access (mHealth) (24, 266, 267). In reproductive and obstetrical healthcare, existing mHealth interventions mainly target weight loss or monitor glucose concentrations (21, 26, 268). Moreover, based on the scientific evidence on the impact of nutrition and lifestyle behaviors (e.g. maternal smoking, alcohol, and folic acid supplement use) on reproduction, and the absence of an mHealth tool to support healthy nutrition and lifestyle behaviors tailored for couples contemplating pregnancy, we developed the online, web-based coaching program called "Smarter Pregnancy" (www.smarterpregnancy.co.uk) (50, 269). This program was first launched in 2011 and developed based on evidence of the effectiveness of nutrition and lifestyle interventions, educational programs using mobile phones (246, 270), our experiences with a Dutch preconception counselling clinic (39, 271), and three theoretical models for behavioral change (251, 272, 273).

In our survey, including more than 2,000 (sub)fertile couples, we already showed that compliance to the regular Smarter Pregnancy program is high (65%). Moreover, we observed a significantly positive association between the improvement of nutrition (intake of fruit and vegetables) and lifestyle behaviors (alcohol consumption and smoking cessation) and pregnancy rate (25, 274). Inherent to the design of a survey, a control group was not included. As a next step towards implementation, we conducted a multicenter single blinded randomized controlled trial to investigate the compliance and effectiveness of Smarter Pregnancy on the improvement of inadequate nutrition and lifestyle behaviors in couples undergoing IVF/ICSI treatment, while pregnancy rate was, amongst others, studied as a tertiary outcome (28).

Material and Methods

Study design and participants

We performed a multicenter, single blinded randomized controlled trial in six IVF centers located in the Netherlands. A detailed protocol of the study has been published previously (28). Briefly, between July 2014 and March 2017, women with an indication for IVF treatment with or without ICSI were informed about the study before their upcoming treatment. Thereafter, they were contacted by a researcher and invited to participate in the trial. Eligible women were between 18-45 years of age, had a sufficient knowledge or understanding of the Dutch language, and were to start their IVF/ICSI treatment within the next three months. Women were excluded in case of oocyte donation or adherence to a specific diet (e.g. vegans). Male partners were also invited to participate unless they were on a diet. All participants gave written and digitally informed consent.

Ethical approval

All procedures involving participants were approved by the Medical Ethical and Institutional Review Board of the Erasmus MC, University Medical Center, Rotterdam, the Netherlands (MEC number NL40414.078.12), and subsequently by all participating centers. The trial was registered with the Dutch Trial Register (NTR4150, http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4150).

Randomization and masking

Participating women were randomly assigned to the intervention (regular version of Smarter Pregnancy) or control group (light version of Smarter Pregnancy) in a 1:1 ratio by computer and stratified according to the study center from which they had been recruited. Permuted blocking ensured that the number of women and men from the different study centers was balanced between the treatment groups. Allocation concealment was used to ensure researchers did not know the order of group assignment at recruitment and randomization. Moreover, researchers were blinded to the allocation of the participants. When a woman and her partner participated together, they both were randomized in the same group.

Intervention

A detailed description of the intervention has been published previously (28). In short, at study entry, all participants completed the short online questionnaire to record baseline characteristics as well as nutritional (vegetables, fruits, folic acid supplement use) and lifestyle (smoking, alcohol) behaviors. Participants assigned to the intervention group subsequently received tailored coaching based on gender, pregnancy status and behaviors identified as inadequate at the baseline screening. At 6, 12, 18, and 24 weeks of coaching, participants were invited to complete a short online questionnaire to monitor changes in their identified risk behaviors and to assess pregnancy status. The results from the questionnaires were used by the algorithm of the program to adjust the content of the coaching program where necessary. The results were presented on a personal online page to show participant's progress and to stimulate compliance. The tailored coaching comprised a maximum of three e-mails or text messages per week which contained tips, recommendations, vouchers, seasonal recipes, feedback on progress, and additional questions addressing pregnancy status, and adequacy of the at baseline identified inadequate behaviors.

Participants assigned to the control group were offered the light version of Smarter Pregnancy. Only at baseline and at 12 and 24 weeks, the participants filled out the same online questionnaire on baseline characteristics and nutritional and lifestyle behaviors, but did not receive feedback on identified inadequate behaviors. Similar to the intervention group, participants of the control group were asked to adjust their pregnancy status every six weeks, if applicable.

To validate the Smarter Pregnancy coaching program at 0, 12 and 24 weeks, blood samples were collected from a subset of participants in the intervention and control group. Samples were kept at -20° Celsius for a maximum of 4 hours (28). The serum was analyzed for folate levels to validate vegetable and fruit intake and use of folic acid supplements. To this end, the haemolysate was prepared by diluting 0.1mL full blood in 0.9mL fresh 1.0% ascorbic acid. After the haemolysate was centrifuged at 1,000g for five minutes at 18°C, serum folate levels were measured using an electrochemiluminescence immunoassay (Modular E170, Roche GmbH, Mannheim, Germany).

A follow-up questionnaire was sent out 12 weeks after completion of the program (i.e. 36 weeks after enrolment), with questions about nutritional and lifestyle behaviors and to record whether or not these behaviors had changed after completing the coaching. Moreover, 52 weeks after the start of the program a follow-up questionnaire was sent out to collect information on whether or not a pregnancy had occurred the last 52 weeks. In case of non-responding, participants were contacted by phone and e-mail.

Outcomes

The primary outcome of the study was improvement of inadequate nutritional behaviors based on a reduction of the dietary risk score (DRS) 24 weeks after starting the Smarter Pregnancy program (25, 39, 271). Vegetable and fruit intake were subdivided into risk scores of 0, 1.5 or 3, where 0 represents an adequate daily intake (vegetables ≥200 grams, fruits ≥2 pieces). A score of 1.5 represents a 'nearly adequate' intake (vegetables 150-200 grams, fruits 1.5-2 pieces). A score of 3 represents an inadequate daily intake (vegetables <150 grams, fruits <1.5 pieces). Folic acid supplement use was considered adequate (score 0) or inadequate (score 3) when the recommended dose of 400 µg per day was either met or not (275). For male participants, folic acid supplement use was not taken into account. The DRS was calculated as the sum of the scores of vegetable, fruit and folic acid supplement intake and ranged from 0 to 9 for women and 0 to 6 for men. A higher risk score reflects more inadequate nutritional and lifestyle behaviors.
Secondary and tertiary outcomes were improvement of nutritional and lifestyle behaviors 36 weeks after starting the Smarter Pregnancy program using the DRS and the lifestyle risk score (LRS) (39, 242). Risk score for smoking was based on average daily use. Because smoking has a profound effect on reproduction, this score carries more weight

than the scores for other risk factors: no smoking (score 0), daily smoking 1-5 (score 1), 6-14 (score 3), ≥15 (score 6) cigarettes. Risk scores for alcohol consumption were based on average weekly use, i.e. no alcohol use (score 0), 1-7 (score 1), 8-14 (score 2), or ≥15 (score 3) alcoholic beverages (glasses) per week. The LRS was calculated as the sum of the scores of smoking and alcohol use and ranged from 0 to 9 for both women and men. Other secondary and tertiary outcomes investigated were the compliance to complete the 24 weeks of the coaching program and the impact of participation as a couple, overweight/obesity, and pregnancy on the primary outcome. Also, cumulative pregnancy rate at 52 weeks after the start of Smarter Pregnancy coaching program was evaluated in both the intervention and control group.

Statistical analysis

The sample size for the trial was based on the estimated reduction in the DRS as primary outcome measure (difference of 0.5 DRS points) in the intervention group compared to the control group (28). Considering alpha=0.05, power=0.80 and a drop-out rate of 10%, we needed to include 1,000 women (2 arms of 500 each) in total.

Compliance was calculated as the percentage of participants who completed the 24 weeks of the Smarter Pregnancy coaching program. Comparison between the intervention and control group was carried out using Chi-square tests. The DRS and LRS were calculated at baseline, after 24 weeks of coaching, and 12 weeks after completion of the program (36 weeks, follow up). Our analyses included all participants who activated the program and either completed the program or resigned prematurely (intention-to-treat analysis). Missing data were handled using the Last-Observation-Carried-Forward (LOCF) method. A linear regression model based on the 'difference in differences' principle was used to analyze differences in improvement of DRS and LRS between groups, adjusted for baseline values of DRS and LRS. The obtained beta coefficient represents the difference in improvement between the intervention and control group. Since participants in the intervention group only received coaching on inadequate behavior, regression analyses were only performed on those participants who showed inadequate behavior at baseline.

Explorative analyses were performed by including an interaction term in the regression model to test whether participation by the male partner, overweight/obesity (body mass index (BMI) ≥25 kg/m^2), or pregnancy influenced the primary outcome. We used a bootstrap method for all analyses because residuals of the linear regression analyses were not normally distributed (276). P-values <.05 were considered statistically significant. We controlled for the probability of type 1 error on a test by test basis. All analyses were performed using Statistical Package for the Social Sciences software (IBM SPSS, Statistics for Windows, version 21.0) and R (R: A language and Environment for Statistical Computing, version 3.1.3 2015 for Windows, R Core Team, Vienna, Austria).

Results

Between July 1, 2014 and March 31, 2017, 988 participants (women and men) were recruited (***Figure 1***). A total of 140 participants withdrew before the start, leaving 848 participants for randomization. The intervention group consisted of 414 participants (308 women and 106 men) and the control group of 434 participants (318 women and 116 men). Baseline characteristics of the study population, stratified by sex, are presented in ***Table 1***. Women in our study had a median age of 33 (IQR 30-36) years and a median BMI of 23.8 (IQR 21.6-27.0) kg/m^2. The median age and BMI of men was 35 (IQR 31-39) years and 25.2 (IQR 23.0-27.8) kg/m^2, respectively. The majority of participants was of Dutch origin and highly educated.

Of the 626 randomized women, 468 completed the program resulting in an overall compliance of 74.8% (211 in the intervention group (68.5%) and 257 in the control group (80.8%), p<.001). Of the 222 randomized men, 176 completed the program resulting in an overall compliance of 79.3% (78 in the intervention group (73.6%) and 98 in the control group (84.5%), p=.045) (***Table 1***).

Supplemental Table 1 shows the distribution of adequate nutritional and lifestyle behaviors among the study population at baseline, 24 weeks and 36 weeks. Both the intervention and the control group show more adequate behavior after 24 weeks of coaching. These findings are supported by the results of the statistical analyses in which is shown that the DRS decreased (i.e. improved) in both the intervention and the control group. However, the decrease of the DRS in the intervention group was significantly larger than in controls (β=0.779, 95%CI 0.456 to 1.090 for women and β=0.826, 95%CI 0.416 to 1.284 for men)

after 24 weeks of coaching (***Table 2; Figure 2***). For women, the decrease of the LRS in the intervention group was also significantly larger than in controls (β=0.108, 95%CI 0.021 to 0.203) (***Table 2; Figure 2***).

Twelve weeks after completion of the program (i.e. 36 weeks after enrolment) the DRS and LRS of participants in both groups were still lower than the baseline scores (***Figure 2***). At 36 weeks after enrolment, the decrease of the DRS compared to the baseline risk scores was larger in the intervention group than in controls (β=0.816, 95%C, 0.478 to 1.142 for women; β=0.639, 95%CI 0.212 to 1.081 for men; ***Table 2***).

Biomarker validation showed that, 12 weeks after enrolment, serum folate levels of women in the intervention group (n=50) were significantly higher than in controls (n=64); median and IQR: 48.6 (28.8-64.1) versus 30.1 (17.9-51.9) nmol/L; ***Supplemental Table 2***). When comparing this subset of participants with the rest of the study population it showed that there were no statistically differences between the groups, apart from the improvement in DRS at the end of the program. Participants in the subset showed larger improvement of the DRS with a median of 1.5 (IQR 1.5-3.0) compared to the remainder of the study population with a median of 0 (IQR 0-1.5).

Analyses of the secondary and tertiary outcomes showed that the results of the women were not significantly influenced by participation of their male partner. It also showed that improvement in nutritional and lifestyle behaviors after 24 weeks of coaching was comparable between overweight/obese and normal weight women. However, subgroup analyses showed that improvement of fruit intake in overweight/obese men was significantly different from that observed in men of normal weight after 24 weeks of coaching (interaction coefficient 0.745, 95%CI 0.167 to 1.312). The regression coefficient (β) for overweight/obese men was 1.001 (95%CI 0.582 to 1.439), whereas for men with normal weight it was five times smaller (β=0.247, 95%CI -0.132 to 0.669; ***Supplemental Table 3***). This was also observed for smoking cessation at 12 weeks after completion of the program (interaction coefficient 0.213, 95%CI 0.010 to 0.541). The regression coefficient (β) for overweight/obese men was 0.141 (95%CI -0.064 to 0.440), whereas for men of normal weight the regression coefficient was negative (β=-0.153, 95%CI -0.623 to 0.001; ***Supplemental Table 4***).

When performing these analyses for pregnancy status we observed a larger improvement in adequate nutritional behavior for pregnant women (β=1.132, 95%CI 0.642 to 1.604) compared to non-pregnant women (β=0.622, 95%CI 0.165 to 1.037; ***Supplemental Table 3***) albeit not significant. Pregnancy significantly influenced lifestyle behavior (interaction coefficient -0.219, 95%CI -0.409 to -0.052). Regression coefficient (β) for pregnant women was 0.135 (95%CI -0.081 to 0.352), whereas for non-pregnant women it was three times higher (β=0.445, 95%CI 0.206 to 0.750; ***Supplemental Table 3***). This was mainly due to smoking cessation (interaction coefficient -0.107, 95%CI -0.255 to -0.001). The regression coefficient (β) for smoking in pregnant women was 0.091 (95%CI 0.001 to 0.306), whereas for non-pregnant women it was three times higher (β=0.248, 95%CI 0.086 to 0.517; ***Supplemental Table 3***). This significant difference was still observed after 36 weeks (interaction coefficient 0.274, 95%CI 0.169 to 0.425).

The pregnancy rates at 52 weeks after start of the coaching program were 62.5% and 67.3% in the intervention and control group, respectively, but was not significantly different between these groups (OR=0.807, 95% CI 0.574 ; 1.134)

Discussion

This multicenter, single blinded, randomized controlled trial demonstrates that the Smarter Pregnancy coaching program is an effective mHealth tool to improve vegetable, fruit and folic acid supplement intakes in particular, and to reduce smoking and alcohol consumption in couples undergoing IVF/ICSI treatment. These effects are most pronounced for intakes of vegetables and fruits, and are supported by higher serum folate levels in the intervention group. In terms of lifestyle behaviors, in the intervention group reduction of smoking was more pronounced in women, whereas reduction of alcohol consumption was more pronounced in men compared to controls.

The high overall compliance to the Smarter Pregnancy coaching program (76%) indicates that participants indeed appreciate this personalized mHealth interventions tailored to a small set of a maximum of five of the most prevalent (vegetables, fruits, alcohol) and strongest (smoking, folic acid supplement use) inadequate behaviors. The compliance in this trial is even higher than shown in our previous survey (65%) and in line with the results of a previous focus group study in which most couples undergoing IVF/ICSI treatment indicated

that they would be interested in tailored intervention programs on the mobile phone (25, 277). Interestingly, compliance to the light version of Smarter Pregnancy program was significantly higher than the regular version. An explanation may be that participants in the intervention group (regular version) are overwhelmed by the intensity of the coaching, making them more likely to withdraw than the controls (light version). This possibility is supported by the fact that more individuals discontinued participation in the intervention group (30.2% versus 18.2%). Nevertheless, the effectiveness of the coaching program for those who maintained participation was still greater for the intervention group.

The finding that the improvement in nutritional behaviors is more pronounced than the improvement in lifestyle behaviors can be explained by the fact that the frequency of inadequate intake of nutrition (on average 73%) and fruits (on average 55%) is much higher than that for smoking (on average 11%). The detrimental effects of smoking and alcohol consumption on fertility and reproductive outcomes are widely acknowledged (106). Therefore, it is to be expected that, in particular, subfertile couples who are willing to stop smoking and drinking alcohol will already have done so. This leaves more room for improvement in the area of nutritional behaviors, the effects of which are unfortunately less widely known in these couples, as substantiated by the high frequency of inadequate vegetable and fruit intakes.

The significant difference in improvement of fruit intake and smoking cessation between normal weight and overweight/obese men was expected as at baseline these men already display more inadequate behaviors than men of normal weight ($p<.01$; data not shown), leaving more room for improvement. This was not apparent in women, which could be due to the limited number of overweight/obese women in our study.This is also inherent to the guidelines of IVF/ICSI treatment in most clinics in the Netherlands, where a maximum BMI is set prior to treatment.

Although we did not show significant differences in pregnancy rates, they were comparable to Dutch data for both the intervention and control group respectively (62.5% and 67.3%) (278). This result might be biased since most people who participate in research are often healthier and higher educated than the general population which makes it a healthy cohort. On the other hand, it indicates that the improvement of

inadequate behaviors following the Smarter Pregnancy coaching program possibly contributes to reproductive health, regardless of using the extended or lean version. This is substantiated by the fact that both the intervention and the control group showed improvement of inadequate behavior.

In a subgroup analysis, pregnant women showed larger improvement of inadequate lifestyle behavior compared to non-pregnant women. This is in line with previous observational studies in which stronger adherence to a healthy dietary pattern and smoking cessation are associated with higher pregnancy rates (279, 280). On the other hand, one may argue that pregnancy renders a women more willing to adopt healthier behavior, as is substantiated by our findings. Although not likely, it could have been possible that pregnant women received counseling regarding a healthy diet and lifestyle separate from the Smarter Pregnancy coaching program, which may have affected our results. At last, women in the intervention group perhaps may have become pregnant at an earlier stage of their treatment. Data on the exact timing of their pregnancy were, however, not available.

Despite evidence of the importance of healthy nutrition and lifestyle regarding reproduction, the low prevalence of adequate fruit and vegetable intake and the high percentage of alcohol consumption in our study group indicates that in the period prior to IVF/ICSI treatment couples continue to make poor lifestyle choices (106, 242, 281-283). This emphasizes that also healthcare providers should take the responsibility to implement nutritional and lifestyle care into preconception and reproductive care. We have demonstrated that one way of achieving this, would be to increase the availability and applicability of the simple, evidence-based mHealth tool Smarter Pregnancy. This is in line with the acceptance of user-friendly and effective mHealth tools in healthcare, in particular supporting patients with specific conditions, such as diabetes and cardiovascular diseases (26, 284). In line with the aforementioned preconception action phases, we have shown that the Smarter Pregnancy coaching program satisfies many of the features of these action phases for a successful implementation in the earliest life course.

Our study has several strengths. Besides the large number of women and men included in this trial, its multicenter design makes the results applicable to various IVF/ICSI settings. Moreover, the results of the self-administered questionnaires are supported with biomarker validation of nutritional behavior by measurement of serum folate, a sensitive marker of short-term folate status. Lastly, the DRS and LRS are validated risk scores based on previous studies.

However, there are also some limitations. Firstly, we did not achieve our estimated sample size of 500 women and 300 men in each group, mainly due to a slower participation rate than expected, which reduced the power to show significance of our secondary and tertiary outcomes. However, differences in effect estimates (betas) between the intervention and control group were still higher than expected and demonstrated a statistically significant effect of the Smarter Pregnancy program regarding the improvement of nutrition and lifestyle behaviors. Secondly, the majority of our study population was highly educated, which may reduce generalization of our findings. Thirdly, the Smarter Pregnancy coaching program was only available in the Dutch language, thereby excluding non-native Dutch speakers, which gives rise to selection bias. The Smarter Pregnancy program has recently become available in the English language (www.smarterpregnancy.co.uk), which means that this limitation has been resolved. Lastly, participants completed self-administered questionnaires, which are susceptible to desirable answers and recall bias. However, they were validated by the biomarkers and we expect that the degree of such bias would be similar between the intervention and control group.

Conclusion

In conclusion, we demonstrated that users of the Smarter Pregnancy coaching program significantly improved inadequate nutritional and lifestyle behaviors. Therefore, we encourage wider implementation of the Smarter Pregnancy coaching program, also in countries other than the Netherlands, to make preconception nutritional and lifestyle care more accessible to patients as well as healthcare providers. Future studies will focus on the effects of improvement of inadequate nutritional and lifestyle behaviors on pregnancy outcomes, such as livebirth, preterm birth, and low birth weight. Last but not least, we would like to emphasize that every approach of improving nutrition

and lifestyle behaviors in an early period of life is an investment that eventually will contribute to the health of current and future generations.

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Table 1. Baseline characteristics and nutritional and lifestyle behaviors of all participating women and men in the multicenter study population (total n=848).

	Women		Men	
	Intervention (n=308)	**Control (n=318)**	**Intervention (n=106)**	**Control (n=116)**
Age (years)	33 (29-37)	33 (30-36)	35 (31-39)	35 (31-41)
BMI (kg/m2)	23.7 (21.6-26.7)	23.8 (21.6-26.3)	25.1 (22.7-26.9)	25.2 (23.2-28.3)
Underweight (<20)	35 (11.4)	31 (9.7)	4 (3.8)	4 (3.4)
Normal (≥20-25)	165 (53.6)	172 (54.1)	46 (43.4)	50 (43.1)
Overweight (≥25-30)	68 (22.1)	81 (25.5)	45 (42.5)	49 (42.2)
Obese (≥30)	40 (13.0)	34 (10.7)	11 (10.4)	13 (11.2)
Missing	0	0	0	0
Geographic background				
Dutch	223 (79.6)	229 (78.7)	84 (95.5)	86 (90.5)
Western	13 (4.6)	21 (7.2)	2 (2.3)	4 (4.2)
Non-western	44 (15.7)	41 (14.1)	2 (2.3)	5 (5.3)
Missing	28	27	18	21
Education				
Low	6 (2.1)	7 (2.4)	4 (4.5)	5 (5.3)
Intermediate	128 (45.7)	92 (32.1)	40 (45.5)	41 (43.2)
High	146 (52.1)	188 (65.5)	44 (50.0)	49 (51.6)
Missing	28	31	18	21
Adequate behavior at baseline				
Vegetable intake	76 (25.5)	90 (28.8)	29 (28.2)	29 (25.7)
Fruit intake	145 (48.7)	139 (45.0)	48 (46.6)	44 (39.6)
Folic acid supplement use	302 (98.1)	310 (97.5)	NA	NA
Adequate dietary risk score (DRS)	49 (19.7)	56 (18.1)	17 (16.5)	12 (10.8)
No smoking	275 (92.3)	286 (93.2)	88 (86.3)	92 (83.6)
No alcohol consumption	192 (64.4)	185 (60.5)	32 (31.7)	30 (27.5)
Adequate lifestyle risk score (LRS)	175 (58.7)	175 (57.2)	27 (26.7)	26 (23.9)
Compliance				
Program completed (24 weeks)	211 (68.5)	257 (80.8)	78 (73.6)	98 (84.5)

Note: values are expressed as median (interquartile range) or as number (%). BMI = body mass index. Dietary risk score = sum of risk scores for vegetable intake, fruit intake and folic acid supplement use. Lifestyle risk score = sum of risk scores for smoking and alcohol consumption.

Table 2. Regression coefficients (β) for the difference in improvement of the individual inadequate behaviors and for the dietary risk score (DRS) and lifestyle risk score (LRS) between the intervention and control group 24 weeks after the start of the program and 12 weeks after completion of the program (i.e. 36 weeks, follow-up), stratified for women and men.

		24 weeks		36 weeks	
		Women	**Men**	**Women**	**Men**
Vegetable intake	β	0.781	0.376	0.620	0.439
	95% CI	0.567 ; 0.973	0.040 ; 0.707	0.415 ; 0.800	0.144 ; 0.737
Fruit intake	β	0.185	0.526	0.245	0.362
	95% CI	-0.026 ; 0.391	0.245 ; 0.833	0.050 ; 0.457	0.061 ; 0.668
Folic acid suppl. use	β	-0.090	NA	0.006	NA
	95% CI	-0.187 ; -0.022		-0.121 ; 0.120	
Dietary risk score	β	0.779	0.826	0.816	0.639
	95% CI	0.456 ; 1.090	0.416 ; 1.284	0.478 ; 1.142	0.212 ; 1.081
Smoking	β	0.090	-0.047	0.065	0.007
	95% CI	0.020 ; 0.184	-0.287 ; 0.110	-0.013 ; 0.167	-0.225 ; 0.151
Alcohol consumption	β	0.037	0.122	0.016	0.055
	95% CI	-0.034 ; 0.111	-0.035 ; 0.302	-0.057 ; 0.091	-0.114 ; 0.242
Lifestyle risk score	β	0.108	0.109	0.067	0.086
	95% CI	0.021 ; 0.203	-0.106 ; 0.300	-0.032 ; 0.165	-0.131 ; 0.277

NA: Not Applicable.
Number of women / men with inadequate behavior at baseline for the different factors; vegetable intake 442 / 156, fruit intake 322 / 122, Folic acid supplement use 14, Dietary risk score 502 / 185, smoking 46 / 33, alcohol consumption 227 / 148, and Lifestyle risk score 254 / 157.

Supplemental Table 1. Adequate nutritional and lifestyle behaviors of the intervention group and the control group at baseline, at the end of the program (24 weeks), and 12 weeks after completion of the program (36 weeks, follow-up).

		Women						Men					
		Intervention (n=308)			Control (n=318)			Intervention (n=106)			Control (n=116)		
		Baseline	**24 weeks**	**36 weeks**	**Baseline**	**24 weeks**	**36 weeks**	**Baseline**	**24 weeks**	**36 weeks**	**Baseline**	**24 weeks**	**36 weeks**
Vegetables, grams per day >200 grams	n	76	124	101	90	89	68	29	40	33	29	31	27
	%	25.5%	41.5%	33.8%	28.8%	28.4%	21.7%	28.2%	38.8%	32.0%	25.7%	27.4%	23.9%
Missing		10	9	9	5	5	5	3	3	3	3	3	3
Fruits, pieces per day >2 pieces	n	145	207	209	139	182	182	48	67	65	44	46	50
	%	48.7%	69.2%	69.9%	45.0%	58.5%	58.5%	46.6%	65.0%	63.1%	39.6%	41.4%	45.0%
Missing		10	9	9	9	7	7	3	3	3	5	5	5
Folic acid supplement use adequate	n	302	298	296	310	314	305	NA	NA	NA	NA	NA	NA
	%	98.1%	96.8%	96.1%	97.5%	98.7%	95.9%						
Missing		0	0	0	0	0	0						
Smoking	n	275	287	246	286	292	288	88	90	80	92	96	95
No smoking	%	92.3%	96.0%	95.0%	93.2%	94.5%	94.4%	86.3%	88.2%	87.0%	83.6%	87.3%	86.4%
Missing		10	9	49	11	9	13	4	4	14	6	6	6
Alcohol consumption	n	192	241	214	185	231	238	32	35	31	30	35	38
No alcohol consumption	%	64.4%	80.6%	80.5%	60.5%	75.0%	78.3%	31.7%	34.7%	32.0%	27.5%	32.1%	34.9%
Missing		10	9	42	12	10	14	5	5	9	7	7	7

Note: n gives the number of participants with adequate behavior, % gives the percentage of participants with adequate behavior

Supplemental Table 2. Serum folate levels of a subset of participants (n=146).

	Intervention		Control	
	Women	**Men**	**Women**	**Men**
	n = 50	n = 11	n = 64	n = 21
Serum folate (nmol/L)				
baseline	35.1 (18.0-45.2)	44.1 (29.4-55.0)	36.3 (23.5-53.6)	35.0 (22.9-39.2)
After 12 weeks	48.6 (28.8-64.1)*	30.6 (20.9-65.7)	30.1 (17.9-51.9)	37.5 (29.9-43.5)
End of program (24 weeks)	35.9 (20.5-41.2)	34.2 (16.9-53.6)	33.9 (18.5-53.0)	40.7 (24.4-57.1)

Note: values are expressed as median (interquartile range)
* P-value <.05

Supplemental Table 3. Regression coefficients (β) for the subgroup analyses of the difference in improvement of the individual behaviors, and the composite dietary risk score (DRS) and lifestyle risk score (LRS) between the intervention and control group 24 weeks after the start of the program, stratified for participation alone or as a couple, for overweight/obese and normal weight, and for pregnancy status.

		Couples		**Women**		**Men**		**Pregnant**	
		Yes	**No**	**Overweight / obese**	**Normal weight**	**Overweight / obese**	**Normal weight**	**Yes**	**No**
		n = 222	n = 404	n = 209	n = 402	n = 113	n = 103	n = 248	n = 344
Vegetable intake	β	0.631	0.702	0.606	0.722	0.216	0.304	0.914	0.527
	95% CI	0.294 ; 0.965	0.469 ; 0.950	0.277 ; 0.952	0.471 ; 0.964	-0.184 ; 0.665	-0.177 ; 0.769	0.566 ; 1.223	0.273 ; 0.791
Fruit intake	β	0.205	0.288	0.325	0.177	1.001	0.247	0.403	0.216
	95% CI	-0.144 ; 0.551	0.043 ; 0.526	-0.017 ; 0.642	-0.064 ; 0.434	0.582 ; 1.439	-0.132 ; 0.669	0.105 ; 0.699	-0.058 ; 0.475
Folic acid suppl. use	β	-0.068	-0.099	-0.194	-0.020	NA	NA	-0.033	-0.084
	95% CI	-0.239 ; 0.001	-0.224 ; -0.011	-0.420 ; -0.072	-0.102 ; 0.054			-0.174 ; 0.001	-0.211 ; 0.017
Dietary risk score	β	0.484	0.934	0.764	0.775	1.070	0.597	1.132	0.622
	95% CI	-0.068 ; 0.942	0.527 ; 1.316	0.233 ; 1.304	0.360 ; 1.158	0.169 ; 1.801	0.029 ; 1.255	0.642 ; 1.604	0.165 ; 1.037
Smoking	β	0.204	0.086	0.083	0.161	0.091	-0.186	0.091	0.248
	95% CI	0.006 ; 0.594	-0.041 ; 0.243	-0.109 ; 0.384	0.035 ; 0.339	-0.174 ; 0.371	-0.645 ; -0.017	0.001 ; 0.306	0.086 ; 0.517
Alcohol consumption	β	-0.009	0.148	0.128	0.062	0.337	0.186	0.025	0.201
	95% CI	-0.239 ; 0.210	-0.015 ; 0.312	-0.112 ; 0.358	-0.089 ; 0.222	0.119 ; 0.575	-0.051 ; 0.467	-0.167 ; 0.197	0.044 ; 0.350
Lifestyle risk score	β	0.190	0.231	0.202	0.195	0.417	0.010	0.135	0.445
	95% CI	-0.122 ; 0.574	-0.012 ; 0.449	-0.111 ; 0.532	-0.007 ; 0.439	0.076 ; 0.747	-0.507 ; 0.329	-0.081 ; 0.352	0.206 ; 0.750

NA: Not applicable

Supplemental Table 4. Regression coefficients (β) for the subgroup analyses of the difference in improvement of the individual inadequate nutritional and lifestyle behaviors, and composite dietary risk score (DRS) and lifestyle risk score (LRS) between the intervention and control group 12 weeks after completion of the program (i.e. 36 weeks, follow-up) stratified for participation alone or as a couple, for overweight/obese or normal weight, and for pregnancy status

		Couples		**Women**		**Men**		**Pregnant**	
		Yes	**No**	**Overweight / obese**	**Normal weight**	**Overweight / obese**	**Normal weight**	**Yes**	**No**
		n = 222	n = 404	n = 209	n = 402	n = 113	n = 103	n = 302	n = 281
Vegetable intake	β	0.392	0.613	0.381	0.610	0.388	0.154	0.511	0.587
	95% CI	0.105 ; 0.711	0.385 ; 0.858	0.088 ; 0.699	0.366 ; 0.843	-0.038 ; 0.816	-0.256 ; 0.529	0.239 ; 0.789	0.311 ; 0.855
Fruit intake	β	0.219	0.383	0.355	0.234	0.774	0.234	0.367	0.370
	95% CI	-0.151 ; 0.564	0.151; 0.619	0.006 ; 0.696	-0.026 ; 0.479	0.334 ; 1.208	-0.158 ; 0.623	0.072 ; 0.647	0.051 ; 0.653
Folic acid suppl. use	β	-0.036	0.013	-0.097	0.045	NA	NA	0.124	-0.104
	95% CI	-0.220 ; 0.124	-0.119 ; 0.151	-0.292 ; 0.051	-0.093 ; 0.187			0.014 ; 0.276	-0.300 ; 0.058
Dietary risk score	β	0.429	1.026	0.697	0.874	0.917	0.376	0.977	0.851
	95% CI	-0.120 ; 0.932	0.614 ; 1.449	0.148 ; 1.245	0.452 ; 1.311	0.250 ; 1.589	-0.260 ; 0.913	0.462 ; 1.464	0.362 ; 1.349
Smoking	β	-0.019	0.095	0.031	0.072	0.141	-0.153	0.080	0.126
	95% CI	-0.269 ; 0.252	-0.036 ; 0.264	-0.231 ; 0.317	-0.076 ; 0.237	-0.064 ; 0.440	-0.623 ; 0.001	0.001 ; 0.250	-0.100 ; 0.414
Alcohol consumption	β	-0.023	0.117	0.056	0.059	0.208	0.138	0.068	0.132
	95% CI	-0.258 ; 0.209	-0.053 ; 0.281	-0.176 ; 0.280	-0.105 ; 0.225	-0.056 ; 0.473	-0.109 ; 0.417	-0.081 ; 0.213	-0.043 ; 0.304
Lifestyle risk score	β	0.026	0.222	0.126	0.118	0.358	0.020	0.203	0.289
	95% CI	-0.350 ; 0.409	-0.044 ; 0.456	-0.249 ; 0.473	-0.125 ; 0.364	0.045 ; 0.699	-0.512 ; 0.367	0.038 ; 0.396	-0.012 ; 0.631

NA: Not applicable

Figure 1. CONSORT flow diagram showing recruitment of participants, exclusions and dropouts.

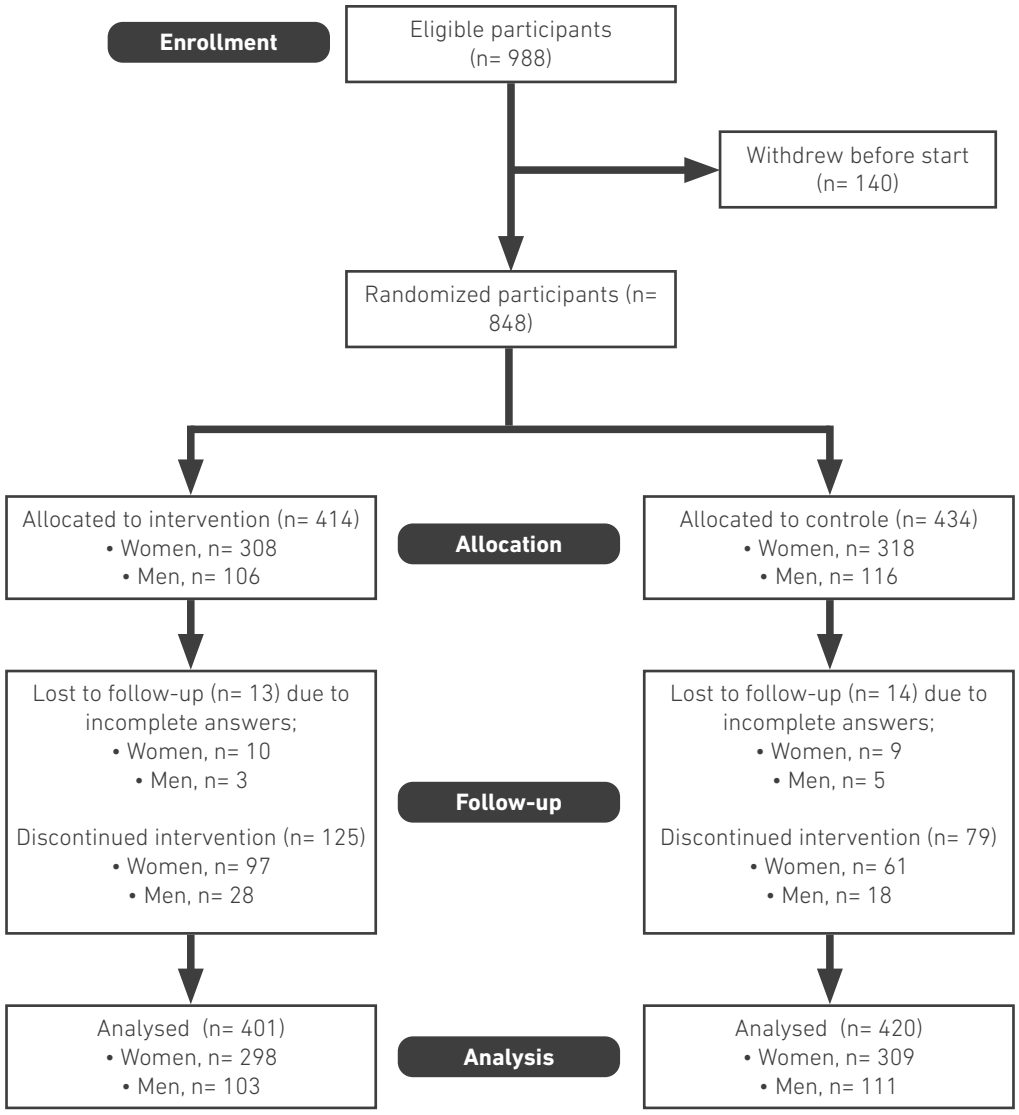
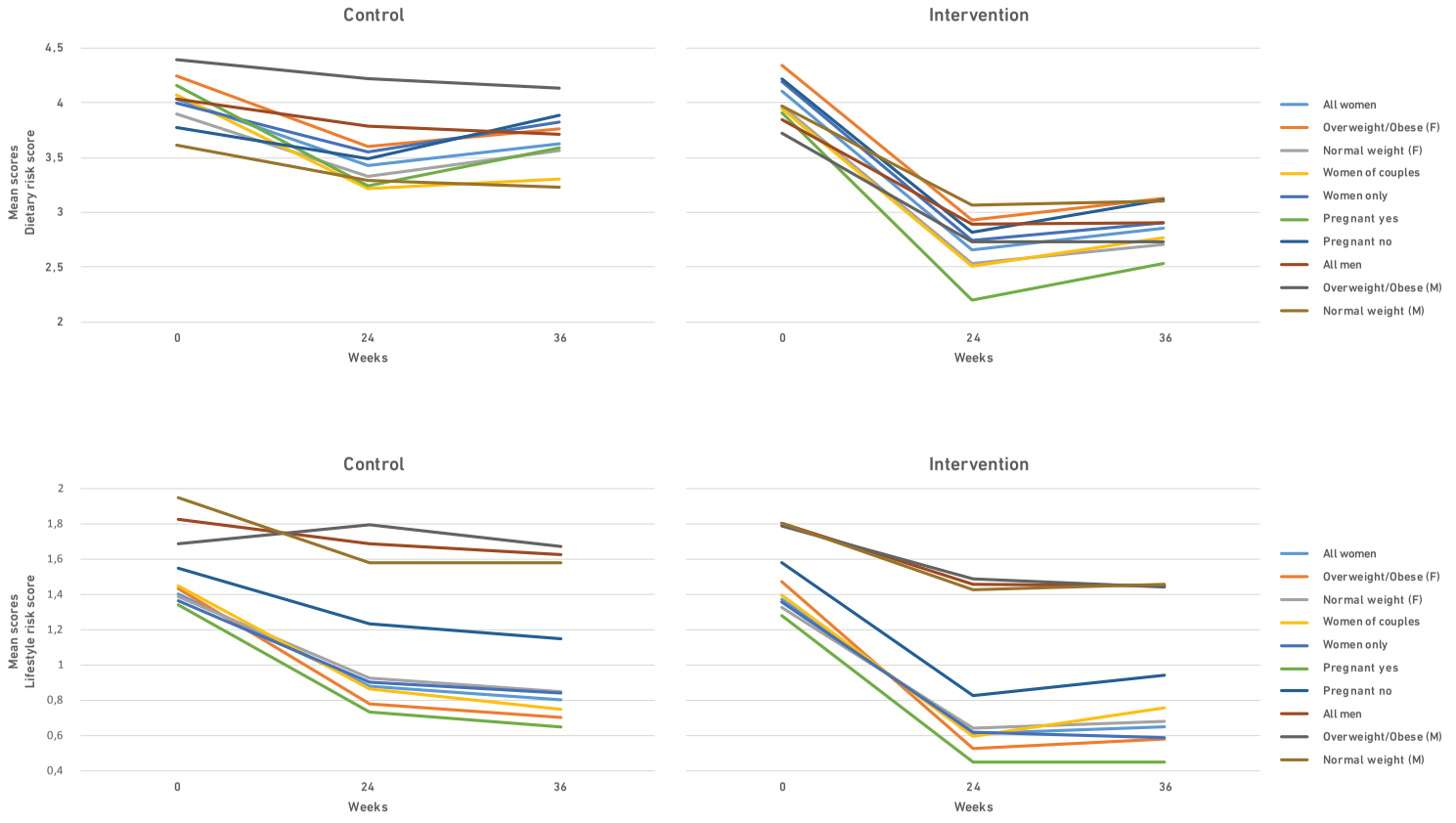


Figure 2. Mean risk scores after 24 weeks (end of the coaching) and 12 weeks after completion of the program (i.e. 36 weeks, follow-up) for Dietary Risk Score (DRS), and Lifestyle Risk Score (LRS).



Note: F = Female. M = Male.

Mobile health coaching on nutrition and lifestyle behaviors for subfertile couples using the Smarter Pregnancy program

model-based cost-effectiveness model

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Elsje C. Oostingh
Robbin H. Ophuis
Maria P.H. Koster
Suzanne Polinder
Hester F. Lingsma
Eric A.P. Steegers
Joop S.E. Laven
Régine P.M. Steegers-Theunissen

Abstract

Background The healthcare costs for reproductive care have substantially increased with the use of in vitro fertilization (IVF) treatment. The mobile health (mHealth) coaching program Smarter Pregnancy is an effective intervention to improve nutrition and lifestyle behaviors and pregnancy rates in (sub)fertile couples, including those who undergo IVF treatment. Therefore, we hypothesize that this mHealth program can also reduce healthcare costs associated with IVF treatment.

Objective This study aimed to evaluate the cost-effectiveness of the mHealth coaching program Smarter Pregnancy and compare it to usual care in women of subfertile couples who start their first IVF cycle.

Methods This model-based cost-effectiveness analysis was performed on data from couples undergoing IVF treatment at the Erasmus MC, University Medical Center Rotterdam. A decision tree model was used to assess the incremental cost-effectiveness ratio (ICER) of ongoing pregnancies and costs of use of the mHealth program as compared to usual care. A probabilistic sensitivity analysis was performed to consider the uncertainty surrounding the point estimates of the input parameters.

Results Based on our model, including 793 subfertile women undergoing IVF treatment, the use of the mHealth program resulted in 86 additional pregnancies and saved €270,000 compared to usual care after two IVF cycles, with an ICER of -€3,050 (95%CI -3,960;-540) per additional pregnancy. The largest cost saving was caused by the avoided IVF treatment costs. Sensitivity analyses showed that the mHealth program needs to increase the ongoing pregnancy rate with at least 51% after two IVF cycles for cost saving.

Conclusions The mHealth coaching program Smarter Pregnancy is potentially cost-saving for subfertile couples preceding their first IVF treatment. Implementation of this mHealth program in routine preconception care for subfertile couples should be seriously considered given the relatively low costs and promising cost-effectiveness estimates.

Introduction

Since the pioneer work of Edwards and Steptoe, in vitro fertilization (IVF) has become an indelible technology in modern era. Although the ongoing pregnancy rate after IVF treatment has tremendously increased (285), subfertility remains a worldwide problem affecting approximately 12% of couples of reproductive age (286). In addition to the medical causes of subfertility, poor nutrition and lifestyle behaviors can impair fertility as well (106). The mobile health (mHealth) coaching program Smarter Pregnancy (260, 287) was developed to motivate (sub)fertile couples to adopt healthy nutrition and lifestyle behaviors. In a survey among (sub)fertile couples and a primary analysis of a randomized controlled trial (RCT) among couples with an IVF treatment indication, we showed that online coaching of participants resulted in significant improvements of their nutrition and lifestyle behaviors (25, 288). Moreover, our survey also showed that improvements in nutritional behavior lead to an increase in ongoing pregnancy rates in fertile and subfertile couples with and without IVF treatment (242). Since the healthcare and societal costs of IVF treatment are substantial (289), and we believe that many costs can be saved when a healthy lifestyle is adopted. Here, we aim to assess the cost-effectiveness of the use of this mHealth program compared to usual care in subfertile women preceding their first IVF treatment.

Methods

Study population

The data were derived from a modelled study population consisting of subfertile women undergoing their first IVF treatment at the Erasmus MC, University Medical Center Rotterdam, the Netherlands. The data of the RCT were used to model nutrition and lifestyle behaviors. In this RCT, participants were randomly assigned to the intervention or the control group. Participants of the intervention group received the complete coaching program and were coached on a maximum of five nutrition and lifestyle behaviors: vegetable-, fruit- and folic acid supplement intake, smoking, and alcohol consumption. Participants of the control group only received a diminished version of the program. At several time points, all participants were asked to fill out questionnaires about their nutrition and lifestyle behavior. In this way, change in behavior could be measured. Participants of the RCT started the program at maximum 2 months before start of their IVF/ICSI treatment, and the program lasts for a period of 24 weeks (***Figure 1***). The

study protocol and primary results of the RCT on the improvement of these behaviors have been published elsewhere (28, 288). In brief, participants in the intervention group showed a significantly larger improvement of inadequate behavior, compared to the control group (288).

Model

A decision tree model was constructed using Microsoft Excel in order to assess the incremental ongoing pregnancies following the first IVF cycle and the costs of the mHealth program as compared to usual care (***Figure 2***). Ongoing pregnancy was defined as a vital pregnancy at 12 weeks of gestation. Women of subfertile couples who underwent their first IVF treatment in 2015 entered the model (n=793). A second IVF cycle was started if the first cycle did not result in an ongoing pregnancy. Pregnancy outcome following the second IVF cycle was the endpoint of the model. This short-term evaluation should therefore be considered a first indication of cost-effectiveness of the mHealth program.

Model scenarios

The usual care scenario reflects usual IVF treatment in the Netherlands. We assumed that all women received an elective single embryo transfer (ET) and that pregnancy rates in usual care are 33% for the first IVF cycle and 23% for the second cycle (290). We furthermore assumed that all women in the intervention scenario were offered the mHealth program (100% coverage). This program was not offered in the usual care scenario (0% coverage). The intervention adherence rate was set at 70%, based on RCT data in which 70% of participants in the intervention group completed the coaching (288).

Model parameters

Analyses were performed from a healthcare and a societal perspective. The healthcare perspective includes costs related to the mHealth program (291), all costs associated with IVF treatment (e.g. laboratory and hospital costs) and other relevant healthcare costs (e.g. general practitioner visits). The societal perspective includes all healthcare costs plus costs outside the healthcare sector (e.g. costs due to absence at work). The model parameters, including their distributions and sources, are reported in ***Table 1***.

Ongoing pregnancy rates after the first and second IVF cycle for the Smarter Pregnancy scenario and the usual care scenario were based on our previous study in the same setting (242) and others (292). A detailed description of the cost calculations has been provided by Fiddelers et al (289). All costs were expressed in euros (€) for the reference-year 2016 based on the Dutch price index (293).

Cost-effectiveness analysis

The primary effect outcome measure was expressed as the number of ongoing pregnancies, after two IVF cycles. Incremental cost-effectiveness ratios (ICERs) from healthcare and societal perspectives were calculated by dividing the difference in costs between the Smarter Pregnancy scenario and the usual care scenario by the difference in the number of ongoing pregnancies in both scenarios. The ICER represents the estimated costs of one additional ongoing pregnancy.

A probabilistic sensitivity analysis was performed to consider the uncertainty surrounding the point estimates of the model input parameters. Probabilistic distributions were assigned to the parameters (***Table 1***). Thereafter, 1,000 model iterations were performed by drawing random values from the distributions assigned to the input parameters. We calculated the average costs and ongoing pregnancies by averaging these 1,000 iterations. We performed deterministic sensitivity analyses to investigate the impact of changing several key parameters of the model: the coverage and adherence rate of the mHealth program and the chance of an ongoing pregnancy following the use of this program.

Results

Based on our model, including 793 women, the mHealth scenario resulted in 369 pregnancies (47%; 95%CI 317;422) and the usual care scenario resulted in 283 pregnancies (36%; 95%CI 209;363) after two IVF cycles (***Figure 1***). The average healthcare costs for the mHealth and the usual care scenario were €6,008,500 (95%CI 5,671,000;6,505,000) and €6,214,800 (95%CI 5,839,500;6,730,300), respectively. The average societal costs for the mHealth and the usual care scenario were €7,492,400 (95%CI 6,821,300;8,369,400) and €7,762,400 (95%CI 7,008,500;8,716,800), respectively (***Figure 3***). The ICERs from healthcare and societal perspectives per additional ongoing

pregnancy equaled -€2,250 (95%CI -3,030;-760) and -€3,050 (95%CI -3,960;-540), respectively. ***Figure 4*** shows that almost all ICERs are located in the southeast quadrant of the cost-effectiveness plane, indicating that use of the mHealth program is cost saving.

The sensitivity analyses (***Table 2***) showed that the mHealth program is cost saving on an average, but the uncertainty surrounding the ICERs increases when the intervention is less effective due to a lower compliance and ongoing pregnancy rate. For example, use of the mHealth program should increase the ongoing pregnancy rate by at least 51% for it to be cost saving compared to usual care when a 70% adherence rate is assumed. Otherwise, given an increased pregnancy rate of 65%, the compliance to Smarter Pregnancy should be at least 49% for it to remain cost saving.

Discussion

Principal findings

This model-based study, based on the estimates of available data, showed that the use of the mHealth program would result in 86 additional pregnancies and a reduction of €270,000 compared to usual care after two IVF cycles, resulting in an ICER of -€3,050 per additional ongoing pregnancy. Sensitivity analyses showed that the use of this mHealth program is cost saving when the ongoing pregnancy rate increases to at least 51% after two cycles of single embryo-transfer IVF treatment.

Strengths and limitations

A strength of our model is the combined use of evidence based data of the population, clinical effectiveness, compliance, and costs to support decision making. Although model parameters would ideally be based on meta-analyses or larger datasets, these were unavailable. Since the Smarter Pregnancy RCT is ongoing, assumptions regarding ongoing pregnancy rates had to be made based on our previous data. In economic evaluations, a time horizon that is long enough to capture all relevant costs and effects is preferred (294). Our study was limited to two IVF cycles, which may be relatively short. However, as the endpoint of our study was to assess the incremental ongoing pregnancy rate, other costs and long term reproductive and health outcomes were not considered.

We evaluated single embryo transfers only, because in the Netherlands, this is the most common IVF strategy. Therefore, costs and ongoing pregnancy rates of other IVF strategies will be different (292).

Comparison with prior work

Several studies have investigated the effectiveness of nutrition and lifestyle interventions preceding fertility treatment. However, most of these studies focus on specific patient groups, such as obese or anovulatory women (295, 296). In accordance with our findings, the study by van Oers et al (297) showed that lifestyle intervention preceding fertility treatment was cost-effective in terms of achieving an ongoing pregnancy within 24 months.

The difference in average societal costs and healthcare costs was relatively small, indicating that the addition of the non-healthcare costs had no substantial impact on the ICER. Because nutrition and lifestyle interventions in preconception care have relatively low additional budget impact, we expect that the chance that the mHealth program is not cost-effective would be low (298).

Conclusions

Our results show that the mHealth coaching program Smarter Pregnancy is potentially cost saving for subfertile couples preceding their first IVF treatment. Although our results are promising, our model requires further validation based on actual data on ongoing pregnancy rates from the Smarter Pregnancy RCT in order to establish the relative cost-effectiveness of the mHealth program with greater certainty. Implementation of the this mHealth program in routine preconception care of subfertile couples should be seriously considered given the relatively low intervention costs and promising cost-effectiveness estimates.

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Conflict of interest

Prof. R.P.M. Steegers-Theunissen is CEO of eHealth Care Solutions (since 2013). No other disclosures were reported.

List of abbreviations

ICER: incremental cost-effectiveness ratio

IVF: in vitro fertilization

mHealth: mobile health

RCT: randomized controlled trial

CI: confidence interval

ET: embryo transfer

Table 1. Model input parameters.

Input parameter	Deterministic value	Probabilistic distribution	Source
IVF[a] costs (per cycle)			
Hospital costs			
Hormone stimulation – medication	€1,580	Fixed	Fiddelers et al. (289)
Hormone stimulation – hospital care	€331	Fixed	Fiddelers et al. (289)
Ovum pick-up	€596	Fixed	Fiddelers et al. (289)
Lab	€1,339	Fixed	Fiddelers et al. (289)
Embryo transfer (ET)	€316	Fixed	Fiddelers et al. (289)
Other	€295	Gamma	Fiddelers et al. (289)
Other healthcare costs			
General practitioner	€3	Gamma	Fiddelers et al. (289)
Other	€13	Gamma	Fiddelers et al. (289)
Costs outside healthcare[b]			
Sick leave	€569	Gamma	Fiddelers et al. (289)
Leave of absence	€141		Fiddelers et al. (289)
Loss of leisure time	€73	Gamma	Fiddelers et al. (289)
Out of pocket expenditures	€77	Gamma	Fiddelers et al. (289)
Informal care	€32	Gamma	Fiddelers et al. (289)
Other	€22	Gamma	Fiddelers et al. (289)
Intervention costs			
Smarter Pregnancy program costs	€61[c]	Gamma	Luyendijk (291)
Lifestyle costs[b]			
Folic acid supplement use	€64	Fixed	Luyendijk (291)
Healthy nutrition	€113	Fixed	Luyendijk (291)
Smoking	€1,223	Fixed	Based on (299) and (300)
Alcohol consumption	€913	Fixed	Based on (301) and (302)
Pregnancy rates usual care			
First IVF cycle	0.329	Beta	Based on Wade et al. (290)
Second IVF cycle	0.229	Beta	Based on Wade et al. (290)
Pregnancy rate intervention			
First IVF cycle – 65% increase	0.543	Beta	Based on Twigt et al. (242)
Second IVF cycle – 65% increase	0.443	Beta	Based on Twigt et al. (242)

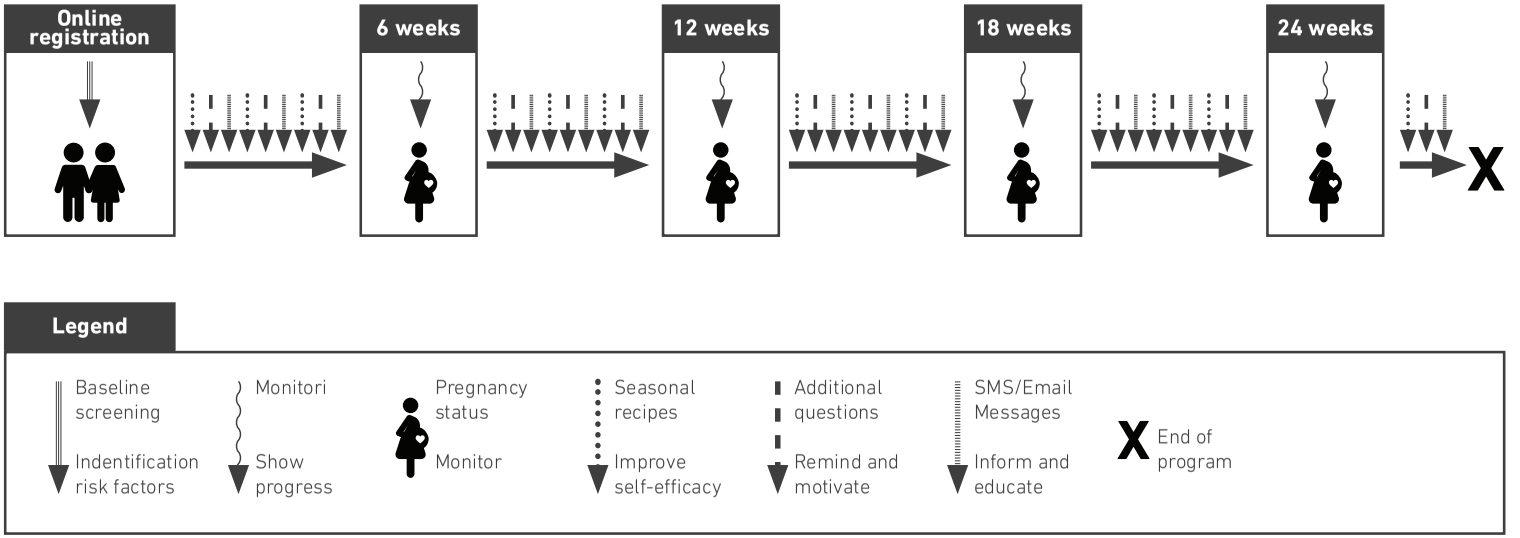
a IVF: In vitro fertilization
b Only included in the analysis from societal perspective. We assumed that the participants who smoke use 10 cigarettes per day (average amount of smokers in the Netherlands) and that alcohol consumers drink one alcoholic beverage per day.
c Based on the annual tariff. This is considered to be an indication for the actual costs, which mainly consist of maintenance, insurance, overhead, and text messages.

Table 2. Results of the sensitivity analyses.

Result	Mean number of incremental pregnancies	Mean incremental societal costs	Mean ICER societal perspective (95%CI)
Main analysis[a]	86	-€270,000	-€3,050 (-3,960;-540)
Sensitivity analyses			
85% intervention compliance	105	-€340,200	-€3,210 (-3,960;-1,630)
55% intervention compliance	63	-€192,000	-€2,840 (-3,920;-120)
45% increase in pregnancy rate (0.477)	64	-€186,300	-€3,070 (-5,610;1,620)
25% increase in pregnancy rate (0.411)	40	-€98,300	-€2,300 (-9,610;9,520)
70% intervention coverage	62	-€187,600	-€2,840 (-3,930;-540)
85% intervention coverage	74	-€227,400	-€2,850 (-3,900;-710)
Worst case scenario[b]	21	-€37,300	-€1,270 (-20,900;13,200)
Best case scenario[c]	123	-€408,900	-€3,600 (-3,900;-1,850)

a 100% intervention coverage, 70% intervention compliance, 65% increase in pregnancy rate
b 70% intervention coverage, 55% intervention compliance, 25% increase in pregnancy rate
c 100% intervention coverage, 100% intervention compliance, 65% increase in pregnancy rate

Figure 1. Overview of both the intervention and control group during their enrollment in the Smarter Pregnancy RCT.



Adapted from M.R. van Dijk et al, The use of the mHealth program Smarter Pregnancy in preconception care: rationale, study design and data collection of a randomized controlled trial. BMC Pregnancy Childbirth. 2017 Jan 26;17(1):46. PMID: 28125970. doi: 10.1186/s12884-017-1228-5.

Figure 2. Decision tree model

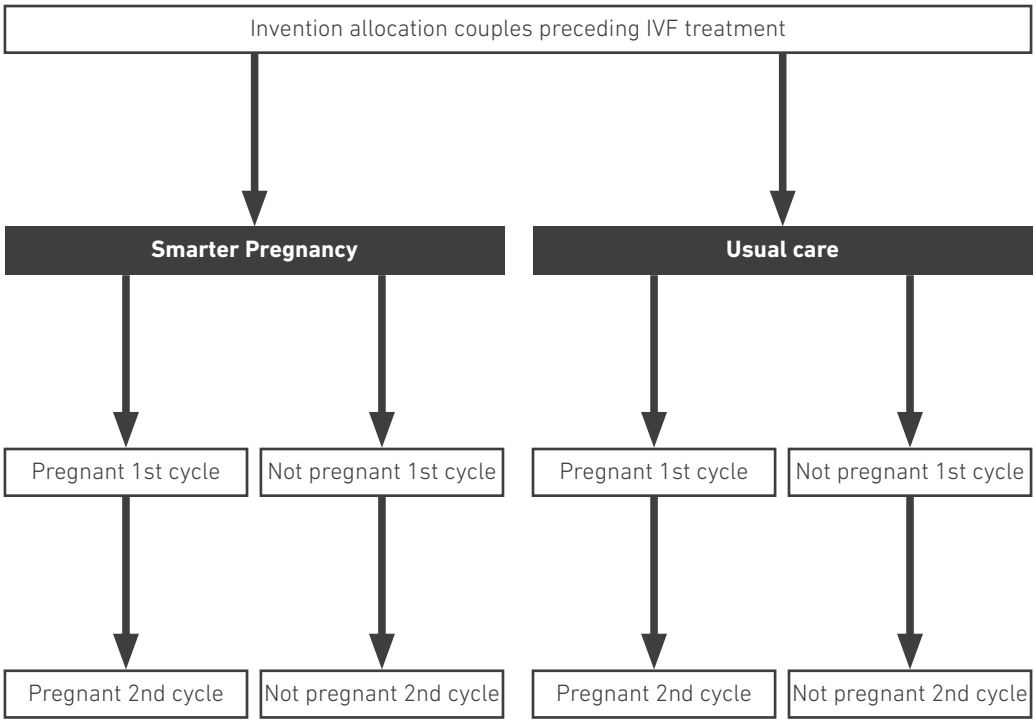


Figure 3. Costs and effects (ongoing pregnancy rate) of the mHealth coaching program Smarter Pregnancy (intervention) and usual care, divided in healthcare and societal perspectives.

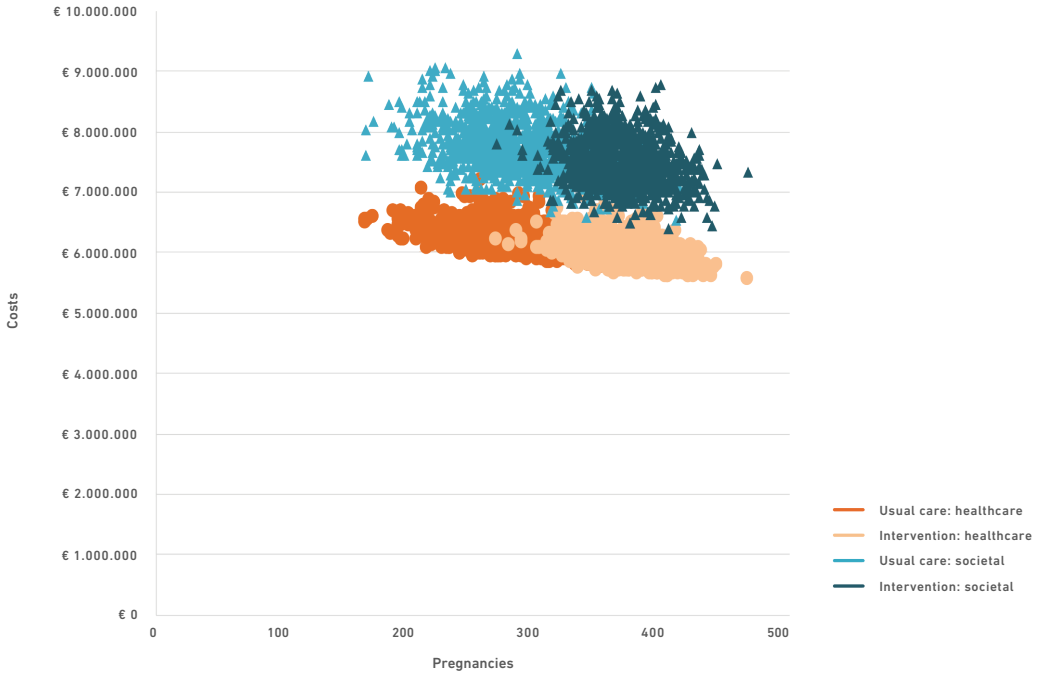
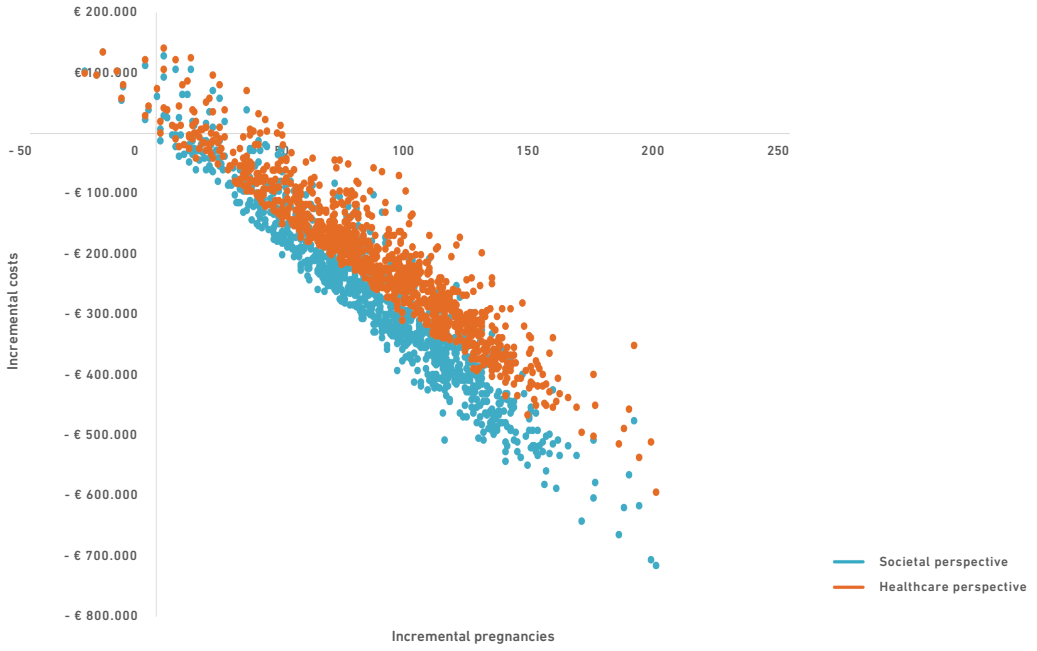


Figure 4.The cost-effectiveness plane



General discussion

The studies in this thesis describe the associations between parental nutrition and lifestyle behaviors and periconception outcomes. Moreover, it was explored to what extend these behaviors can be improved through the mHealth intervention Smarter Pregnancy. In this chapter the methodological considerations, main findings and clinical implications of these studies are discussed. Finally, recommendations for future research are provided

Cohort studies versus clinical trials

The studies described in this thesis have been conducted in two prospective observational cohort studies, namely the Rotterdam periconception cohort (Predict study) and the preconception outpatient clinic 'Achieving a healthy pregnancy', and in one randomized controlled trial (RCT), called the Smarter Pregnancy clinical trial (28, 29, 39). These studies were all designed to investigate associations between periconceptional parental health determinants, reproductive performance and pregnancy course and outcome.

Although a prospective cohort study is considered the gold standard for observational studies, inherent to the study design, causality cannot be demonstrated due to potential residual confounding. Our findings should therefore be considered with caution (303). The strength of cohort studies however, is that they can be used to identify associations between risk factors and diseases. In both cohort studies described in this thesis, data were prospectively and longitudinally collected from the periconception period onwards, which reduced the chance of recall bias and provided us with valuable data on a critical time span in reproductive life. This is exactly what cohort studies are about; they provide us with information about the life histories of populations and the individuals who comprise them (304). However, the fact that patients in both cohorts were included in one single tertiary hospital increases the internal validity of the estimates of a high risk population but limits the external validity of our results to the general population.

Since the introduction of RCTs in clinical medicine, by evaluating treatment of tuberculosis with Streptomycin (305), this design is now regarded the gold standard to evaluate the efficacy of an intervention or therapy intended to improve outcome. The studies described in the second part of this thesis were conducted in the Smarter Pregnancy clinical trial, in which the effectiveness of the mHealth coaching program Smarter Pregnancy was investigated. This experimental study was designed as a RCT in which the common strengths of this design (i.e. the development of a prospective

study protocol with strict inclusion and exclusion criteria, a well-defined intervention, and predefined endpoints) were taken into account (306). Selection bias was minimized by randomly allocating participants to either the intervention or the control group. However, it is to argue whether selection bias could still have occurred, since patients who agreed to participate in this trial may have been more motivated to change their behavior compared to patients who denied participation.

One may argue that conducting this RCT in couples preceding IVF/ICSI treatment is sub-optimal as it can be expected that those patients are more aware of the detrimental effects of inadequate nutrition and lifestyle behaviors on fertility and have therefore already changed such inadequate behaviors. However, in this thesis we have shown that a high percentage of these women and men still have inadequate nutrition and lifestyle behaviors, so this argument can be refuted.

As the Smarter Pregnancy RCT was performed in multiple IVF centers throughout the Netherlands, our results are generalizable to all patients preceding IVF/ICSI treatment. However, the validity of our results to couples that undergo other kinds of subfertility treatments, such as ovulation induction or intrauterine insemination, remains unknown and should be further investigated. On the other hand, as shown in a previous survey and RCT among the general population, participants of Smarter Pregnancy also improved nutrition and lifestyle behaviors and showed high compliance and usability which indicates that Smarter Pregnancy can be widely used (25, 307).

In the end, all types of evidence rely primarily on the stringency with which studies are performed and the care with which the results are interpreted, so none of the study designs should be considered separately (306).

Assessment of nutrition and lifestyle behaviors

Since the human diet is complex and varies extensively among individuals, studying dietary intake is challenging. For example, people tend to consume meals that consist of a varying combination of foods instead of single nutrients. Moreover, biological interactions and high correlations exist among nutrients (308). To address these issues, studying dietary patterns instead of single nutrients is most adequate since this reflects

the overall nutritional intake of participants, thereby covering a range of nutritional factors (145). To perform dietary pattern analyses, we used principal component analysis (PCA), which is considered the state-of-the-art statistical method in nutritional epidemiology. The benefit of this data-driven method is that a pre-specified hypothesis on associations between single nutrients or food groups and the outcome of interest is not necessarily required (154). In both cohort studies described in this thesis, participants completed a validated self-administered food frequency questionnaire consisting of 196 questions, to obtain data on nutrition behaviors. However, in clinical practice such extensive questionnaires are not practically feasible. Therefore, in the Smarter Pregnancy clinical trial, we focused on the main components of nutrition and lifestyle behavior (i.e. intake of fruit, vegetables, folic acid supplement use, smoking and alcohol) and composed a validated dietary and lifestyle risk score (DRS, LRS respectively) based on previous studies (39, 271). Herewith, we provided questionnaires on these behaviors which are clinically applicable and can be widely used.

Maternal impact on periconception outcomes

It is widely acknowledged that the nutritional status and health of women before conception and during pregnancy has profound implications for the growth, development, and long-term health of their offspring (309). The former is mostly known as the "Developmental Origins of Health and Disease" theory (DOHaD). In the last few years this paradigm is gradually shifting towards the preconception period, which is considered the most critical time span for optimizing gamete function and early placental development. This is supported by results from the Dutch Famine birth cohort study in which is shown that maternal under-nutrition during gestation, especially in first trimester, is associated with increased risk of cardiovascular and metabolic disease in offspring (14). This theory of environmental epigenetic transgenerational inheritance is further substantiated by other studies showing for example, that maternal overnutrition is associated with diminished embryo developmental potential due to excess metabolites such as insulin, triglycerides, and leptin (310). On the other hand, a recent animal study in mice fed with low protein diet showed diminished brain development in offspring (311, 312). Maternal malnutrition results in reduced circulating concentrations of insulin and amino acids which change the metabolite milieu of the direct environment of the embryo (310). Moreover, it negatively affects angiogenesis and vasculogenesis leading

to placenta malproliferation (313). The studies described in this thesis substantiates the aforementioned hypotheses by showing that maternal modifiable nutrition and lifestyle behaviors are associated with fecundity and other periconception outcomes such as miscarriage, time to pregnancy and embryonic growth. Herewith underlining the need for optimizing preconception health. Thereafter, the most important next step is to reach women of reproductive age.

In this modern technological era, patients feel comfortable using the Internet and mobile applications to provide themselves with information regarding healthcare, rather than consulting a healthcare professional (277, 314). This implicates that healthcare in general should make more use of digital programs, such as mHealth, in addition to usual care. Smarter Pregnancy is an example of such mHealth tool; one that is easily accessible and applicable in clinical practice, as confirmed by a 75% compliance (315). We also showed that Smarter Pregnancy is an effective coaching program as participating women and men significantly improved their intake of fruit and vegetables and reduced their smoking and alcohol consumption. Moreover, as cost-effectiveness also is an important factor for the usability and implementation of an intervention, we showed that Smarter Pregnancy is potentially cost saving from both a societal as well as a health care perspective as it yields more ongoing pregnancies against lower costs compared to usual care.

These interventions can help to create awareness and stimulate behavioral change, but the next challenge is how to support people in sustaining these behavioral changes. One way to achieve this goal is to not only tailor these interventions to specific target groups with high-risks profiles such as diabetes, cardiovascular disease or former pregnancy complications, as nowadays most interventions do (284). But instead, it should also be more tailored to the general population, thereby increasing the use of mHealth applications and thus increasing the uptake of information. By tailoring applications as much as possible so they take into account differences between populations (e.g. socioeconomic status), we take into account that differences in education and income for example, often leads to differences in beliefs, and subsequently leading to differences in changing behaviors (316). Thereby making sure that every individual can be convinced of the health gain of a healthy preconception period.

Paternal impact on periconception outcomes

Whilst the connection between maternal nutrition and lifestyle behavior and the long-term health of her offspring has been studied in detail, links between paternal nutrition and lifestyle behavior and embryogenesis and offspring health are scarce but emerging (178). Different mechanisms through which paternal inheritance is carried out are identified. First, sperm epigenome has previously shown to be affected by paternal overnutrition and obesity (317). Possibly, dietary disturbance of the one carbon metabolism to supply methyl groups for DNA and histone methylation can lead to these epigenome changes (318). Epigenetic inheritance seems the likeliest candidate to carry paternal information to offspring, by cytosine methylation, chromatin structure, and RNA (319). Second, seminal plasma composition and seminal fluid are found to impact amongst others, embryonic development, maternal uterine environment, placental size, and offspring health (9). Lastly, sperm motility can be affected by paternal conditions and by altering the position within the fallopian tube where the fertilization occurs, it could thereby affect offspring phenotype (319). By, amongst others, showing that strong adherence to a healthy dietary pattern was positively associated with semen quality, the studies described in this thesis underline the aforementioned paradigm that paternal nutrition and lifestyle behavior affects spermatogenesis and embryogenesis and subsequent may have an enduring legacy across the lifespan of offspring health. This is of sufficient clinical importance to prompt a call for preconception health for both partners preconceptionally (280).

As shown by a focus group study, men often feel branched at the sideline since women are considered the center of attention during pregnancy (320). As said, this point of view has to be shifted more towards men as well as to the importance of the period preconceptionally. A first step to achieve this goal would be by creating motivational information, which increases the awareness of the paternal influences on their offspring and on increased success of maternal lifestyle interventions, in the end hopefully leading to behavioral change if necessary (25, 321). Hereby making use of the so-called fatherhood identity theory, which suggest that men undertake a significant shift in self-identity when considering themselves to be fathers (322). Enabling men to sustain their behavioral change, online platforms such as Smarter Pregnancy can be of use. Interactive aspects such as serious gaming could be incorporated, and can be of help to make these programs even more sustainable.

Conclusion and future perspectives

The findings presented in this thesis support the accumulating evidence of the impact of unhealthy parental nutrition and lifestyle behaviors on periconception outcomes and the lifetime health of the future child. Moreover, we demonstrated that the prevalence of modifiable inadequate nutrition and lifestyle behaviors is still very high among couples contemplating pregnancy, which is in line with other studies (39, 282, 323). This indicates that there is still a lot to gain and that health care professionals should be more encouraged to educate their patients about the known detrimental effects of unhealthy nutrition and lifestyle behaviors on periconception outcomes, for example through adequate preconception care (PCC) (260, 287, 324-326). In other words, with the studies described in this thesis we aimed to convince professionals and parents to be about the importance of the preconception period and to provide them with a useful evidence based (cost) effective tool to change any inadequate nutrition or lifestyle behavior.

PCC is defined as a set of interventions that aim to modify biomedical, behavioral, and social risks to parental health and the health of their future child (21). PCC thereby provides a window of opportunity to timely eliminate potential risk factors for subfertility and adverse pregnancy outcomes. The importance of the shift towards the preconception period is supported by the studies performed in this thesis. The described studies underline the need for extension of PCC towards more attention for improvement of inadequate nutrition and lifestyle behaviors. Moreover, we have to shift our focus more towards men and their contribution to reproductive outcomes, as we have shown in this thesis that nutrition and lifestyle behaviors of men impact his semen quality and subsequent embryogenesis and placentation.

With the studies performed in this thesis we also aimed to contribute to more awareness about the importance of healthy preparation for pregnancy and thereby lowering barriers for the uptake of PCC for both couples trying to conceive as well as their healthcare providers (327). To ensure that this knowledge will be widely supported, it is of utmost importance that we already start building this foundation before reproductive age, for example at adolescent age (309). In this way, adopting healthy nutrition and lifestyle behaviors and subsequently preparing yourself to achieve a healthy pregnancy

will be common sense. Making people more aware and carefully thought through the importance of healthy nutrition and lifestyle behaviors is also necessary since usual health care currently provides couples with early prenatal care, i.e. health care during the first trimester, but this is often too late as most fetal organs have already been formed before the 10th week of gestation. By the time a couple have their first early prenatal visit, interventions to prevent birth defects or adverse outcomes come too late to have any effect, this indicates the importance for health care preconceptionally (23). Couples experiencing subfertility are a specific target group often acceptable for advice. Subfertility clinics should therefore invest more to address the importance of healthy nutrition and lifestyle behaviors prior to treatment. For example, by making counseling by a dietician mandatory before treatment or by even denying treatment to patients who refuse to quit smoking or consume alcohol.

Future studies should focus on unifying and standardizing periconception outcomes and measurements in order to collect data for a robust meta-analysis to calculate risk ratios. Combine data and participate in data sharing as recently performed in the PrePARED consortium is of major importance (328). Furthermore, causal pathways should be investigated in more detail.

In conclusion, by continuing to establish associations between nutrition and lifestyle behaviors and periconception outcomes and by elucidating the exact underlying mechanisms, couples that are trying to conceive may be more motivated to adopt healthier nutrition and lifestyle behaviors. Moreover, this opens up possibilities to develop (personalized) interventions that enable women and men to adopt and sustain such healthy behaviors. The randomized controlled trial we performed with Smarter Pregnancy set an example for developing similar research programs in order to expand the availability of qualitative and reliable health care programs. In an ideal world, an integrated online platform with information regarding the periconception period but also pregnancy course, newborns, and even the first years of a child's life would probably be most effective.

Summary

Samenvatting

In the 1980's David Barker developed the nowadays well-known 'Thrifty phenotype hypothesis' (329). With this hypothesis it was suggested that the fetus adjusts its biology to the signals received from the mother, thereby readying itself to the world it is about to enter (330). This hypothesis revealed new scientific insights suggesting that maternal nutrition and lifestyle behaviors during pregnancy have impact on health in later life (20, 50). As many adverse birth outcomes originate in the periconception period, nowadays scientists are more and more interested in the association between modifiable lifestyle factors preconceptionally and periconception outcomes such as fertility, time to pregnancy, miscarriage, embryonic and early placental growth and development (5). Several risk factors for impaired fertility and pregnancy outcomes have been found, however conclusive summaries are scarce. The main aim of this thesis was to investigate the impact of nutrition and lifestyle behavior on periconception outcomes and to what extend these behaviors can be improved by mHealth coaching. In the introduction we provided the background for this thesis (***Chapter 1***).

In ***Part I*** of this thesis we investigated the association between parental nutrition and lifestyle behaviors and periconception outcomes. In ***Chapter 2***, we conducted a systematic review of 49 observational studies, providing an important overview of maternal modifiable lifestyle factors (i.e. smoking, alcohol consumption, folic acid and/or multivitamin supplement use, BMI, and physical activity) and their impact on fecundity and other periconception outcomes such as fertility, miscarriage and embryonic growth. The results of this systematic review demonstrate that fecundity is negatively impacted by smoking, alcohol use and poor diet. Increasing BMI was associated with a prolonged time to pregnancy and an increased risk of first-trimester miscarriage. This increased risk was also found when consuming alcohol and caffeine, vitamin supplement use however showed a decrease in this risk. Moreover, the underlying mechanisms of the negative impact of smoking, alcohol consumption, and caffeine on periconception outcomes were addressed, as well as the positive impact of healthy nutrition and vitamin supplement use. Herewith, we emphasized the importance of healthy nutrition and lifestyle behaviors and contributed to more awareness on this subject.

For a long period of time, the impact of maternal factors on reproductive outcome was the only focus in research, leaving paternal impact out of sight. As studies showed that

besides oogenesis, spermatogenesis is also subject to epigenetic alterations, studying paternal influences on semen parameters and fertility gained more attention. At fertilization, not all epigenetic marks are removed from the genome of gametes, allowing the possibility of transgenerational inheritance (179). This epigenetic information is carried forward by three major carriers which are known to be influenced by nutrition (179, 319). Therefore, in ***Chapter 3*** the association between nutrition and semen quality was studied. We here showed that paternal strong adherence to a healthy dietary pattern positively influences several semen parameters e.g. sperm concentration (β 0.28; 95% CI,0.11–0.44), total sperm count (β 1.37; 95% CI, 0.24–2.50), percentage progressive motile sperm (β 4.31; 95% CI, 0.68–7.94), and total motile sperm count (β 0.32; 95% CI, 0.11–0.53). Our findings add to the existing knowledge that healthy dietary patterns rich in intake of fruit, vegetables, fish, and whole grains, have a beneficial effect on semen parameters as these food groups are sources of antioxidants and polyunsaturated fats (PUFA) (173, 331).

With the associations between the aforementioned environmental factors and semen quality, the question raised whether this can be extended to embryonic growth, measured by crown-rump length, and embryonic volume. In contrast to the well-known impact of maternal nutrition and lifestyle behaviors on embryonic growth (332), we were not able to demonstrate the impact of paternal nutrition on embryonic growth (***Chapter 4***). This might be due to the fact that the impact of maternal dietary patterns overrules the impact of the male partner. However, literature on this topic is scarce, revealing a new scientific field yet to discover.

In ***Chapter 5*** we studied the association between sympathomimetics, which is an often used medication indicated for treatment of asthmatic disease, and semen quality, since pharmacological interventions are also known to potentially modify epigenetic information (333). We showed that use of sympathomimetics is positively associated with a 10% higher sperm motility (β 10.27; 95% CI, 3.26-17.27). Moreover, in this study we found that in men with normospermia (i.e. a total motile sperm count of ≥10 million spermatozoa), the use of sympathomimetics is positively associated with sperm concentration (β 0.30; 95% CI, 0.032-0.57).

In ***Part II*** of this thesis, we investigated the mHealth coaching program Smarter Pregnancy which was developed to empower couples contemplating pregnancy to adopt healthier nutrition and lifestyle behavior. We performed a randomized controlled trial among couples undergoing an IVF/ICSI treatment. The RCT was conducted from 2014 until 2017, and included 662 women and 222 male partners. The rationale and study design are presented in ***Chapter 6***.

In ***Chapter 7***, the effectiveness of this mHealth coaching program was demonstrated by evaluating the results of the randomized controlled trial. Overall, it showed that Smarter Pregnancy is an effective tool as participants in the intervention group showed larger improved of inadequate behavior compared to participants in the control group (β=0.78, 95%CI 0.46-1.09 and β=0.83, 95%CI 0.42-1.28) for women and men, respectively). The largest improvements were made in nutritional behaviors; for women in both vegetable and fruit intake and for men in fruit intake. Regarding improvement in lifestyle behaviors, women were more likely to quit smoking and men were more likely to lower alcohol consumption. Analysis of the nutrition and lifestyle behaviors twelve weeks after the coaching had ended, showed that that the improvements in those behaviors were still present, indicating that the accomplished behavioral changes did not only lasts during the coaching period, but also remained thereafter. It has to be studied however, how the improvements in nutrition and lifestyle behaviors will impact fertility and pregnancy outcome.

In intervention studies, cost-effectiveness is, besides reliability, quality and effectiveness, an important determinant of the success of an intervention. Therefore, in ***Chapter 8*** we studied the cost-effectiveness of the mHealth coaching program Smarter Pregnancy by using model-based scenarios. This study showed that Smarter Pregnancy is potentially cost saving from both a societal as well as a healthcare perspective as it yields more ongoing pregnancies against lower costs compared to usual care (ICER -€3,05 (95%CI -3,96;-539) per additional pregnancy).

Summarizing, in this thesis the evidence of the importance of healthy nutrition and lifestyle behavior during the preconception period is substantiated. Moreover, we demonstrated that the prevalence of modifiable inadequate nutrition and lifestyle

behaviors is still very high among couples contemplating pregnancy. However, we also showed that the mHealth coaching program Smarter Pregnancy is an effective tool to improve inadequate behavior. Overall, with this thesis we aimed to increase the awareness about the importance of the preconception period, thereby lowering barriers for uptake of preconception care for both couples who are trying to conceive as well as healthcare professionals. Moreover, we have to shift our focus more towards men as we have shown in this thesis that his nutrition and lifestyle behavior impact semen quality and subsequent embryogenesis and placentation. To conclude, improving nutrition and lifestyle behavior preconceptionally is an investment that eventually will contribute to the health of current and future generations.

Rond het jaar 1980 ontwikkelde David Barker zijn welbekende Dohad-theorie waarin wordt gesteld dat het ongeboren kind zich in de baarmoeder al aanpast aan de situatie in de buitenwereld via de signalen die hij van zijn moeder krijgt, zichzelf op deze manier klaarmakend voor de wereld die hij binnenkort zal betreden (329, 330). Deze hypothese onthulde een nieuw wetenschappelijk inzicht, namelijk dat de voeding en leefstijl van de moeder gedurende de zwangerschap de gezondheid van het kind op latere leeftijd beïnvloedt (20, 50). Aangezien de oorsprong van veel negatieve zwangerschapsuitkomsten in de periconceptieperiode ligt, zijn wetenschappers tegenwoordig steeds meer geïnteresseerd in de associatie tussen aan te passen leefstijl gewoontes en periconceptionele uitkomsten zoals vruchtbaarheid, tijd tot zwangerschap, miskraam, embryonale groei en vroege placentatie (5). Verschillende risicofactoren voor verminderde vruchtbaarheid en negatieve zwangerschapsuitkomsten zijn gevonden, echter duidelijke overzichten blijven schaars. Het belangrijkste doel van dit proefschrift was zodoende het onderzoeken van de invloed van voeding en leefstijl gewoontes op periconceptionele uitkomsten en tot in welke mate deze gewoontes kunnen worden verbeterd middels mHealth coaching. In de introductie wordt de achtergrond van dit proefschrift beschreven (***Hoofdstuk 1***).

In ***Deel I*** van dit proefschrift hebben we de associatie tussen de voeding en leefstijl van ouders en periconceptionele uitkomsten onderzocht. In ***Hoofdstuk 2*** hebben we een systematisch literatuuronderzoek uitgevoerd met 49 studies wat een belangrijk overzicht heeft gegeven van aan te passen leefstijl gewoontes (roken, alcohol gebruik, gebruik van foliumzuur en/ multivitaminen, BMI en lichamelijke activiteit) en de invloed hiervan op periconceptionele uitkomsten zoals vruchtbaarheid, miskraam en embryonale groei. De resultaten van dit literatuuronderzoek laten zien dat vruchtbaarheid negatief wordt beïnvloedt door roken, alcohol gebruik en een ongezond voedingspatroon. Een hoger BMI is geassocieerd met een langere tijd tot zwangerschap en met een hoger risico op miskramen. Dit verhoogde risico op miskramen werd ook gezien bij het gebruik van alcohol en cafeïne, het gebruik van vitaminen daarentegen zorgde voor een lager risico. Daarnaast werden in dit hoofdstuk de onderliggende mechanismen van de negatieve invloed van roken, alcohol en cafeïne op periconceptionele uitkomsten beschreven, alsook de positieve invloed van een gezond voedingspatroon en vitaminen gebruik. Hiermee hebben we het belang van gezonde voeding en leefstijl benadrukt en bijgedragen aan toegenomen bewustwording over dit onderwerp.

Lange tijd was de invloed van moeder op reproductieve uitkomsten de enige focus in wetenschappelijk onderzoek, daarbij de invloed van vader buiten beschouwing latend. Echter, sinds studies hebben aangetoond dat behalve de oögenese, de spermatogenese ook onderhevig is aan epigenetische veranderingen, wordt er steeds meer onderzoek gedaan naar de invloed van vader op semenkwaliteit en voortplanting. Tijdens de bevruchting wordt niet alle epigenetische informatie verwijderd uit het genoom van de gameten, waardoor er transgenerationele overerving plaats kan vinden (179). Deze epigenetische informatie wordt overgedragen middels drie mechanismen welke worden beïnvloedt door voeding (179, 319). Zodoende hebben we in ***Hoofdstuk 3*** de associatie tussen voeding en semenkwaliteit onderzocht. We lieten zien dat wanneer vader een gezond voedingspatroon had dit verschillende semenparameters positief beïnvloedde, namelijk semen concentratie (β 0.28; 95% CI,0.11–0.44), totaal aantal zaadcellen (β1.37; 95% CI, 0.24–2.50), percentage progressief beweeglijke zaadcellen (β 4.31; 95% CI, 0.68–7.94), en VCM (β 0.32; 95% CI, 0.11–0.53). Onze resultaten dragen bij aan de bestaande kennis dat een gezond voedingspatroon wat rijk is aan fruit, groente, vis en volkoren producten, een positief effect heeft op semenkwaliteit doordat het rijk is aan anti-oxidanten en onverzadigde vetzuren (173, 331).

Gezien de gevonden associatie tussen de hiervoor genoemde omgevingsfactoren en semenkwaliteit, rees de vraag of dit ook zou gelden voor embryonale groei, gemeten middels kruin-staart lengte en embryonaal volume. In tegenstelling tot de welbekende invloed van voeding en leefstijl gewoontes van moeder op embryonale groei (332), konden wij helaas geen invloed van voeding en leefstijl gewoontes van vader op embryonale groei aantonen (***Hoofdstuk 4***). Dit heeft mogelijk te maken met het feit dat de invloed van het voedingspatroon van moeder de invloed van de vader overstemt. Echter, literatuur over dit onderwerp is zeldzaam wat een nieuw wetenschappelijk onderwerp naar voren brengt waar nog veel over valt te ontdekken.

In ***Hoofdstuk 5*** hebben we de associatie tussen sympathicomimetica, een veelgebruikt medicament voor astma, en semenkwaliteit onderzocht, aangezien het bekend is dat farmacologische interventies mogelijk de epigenetische informatie bewerken (333). We lieten zien dat het gebruik van sympathicomimetica positief is geassocieerd met een 10% betere beweeglijkheid van het zaad (β 10.27; 95% CI, 3.26-17.27). Daarnaast

hebben we gevonden dat in mannen met een normospermie (VCM van ≥10 miljoen spermatozoa), het gebruik van sympathicomimetica positief is geassocieerd met semen concentratie (β 0.30; 95% CI, 0.032-0.57).

In ***Deel II*** van dit proefschrift hebben we het mHealth coaching programma 'Slimmer Zwanger' onderzocht wat was ontworpen om koppels die proberen zwanger te raken te stimuleren gezonde voeding en leefstijl gewoontes aan te nemen. We hebben een gerandomiseerd onderzoek (RCT) uitgevoerd onder koppels die een IVF/ICSI behandeling zouden ondergaan. Deze RCT werd uitgevoerd tussen maart 2014 en juli 2017, en er werden 662 vrouwen en 222 mannelijke partners geïncludeerd. De rationale en het ontwerp van de studie worden beschreven in ***Hoofdstuk 6***.

In ***Hoofdstuk 7*** wordt de effectiviteit van dit mHealth coaching programma beschreven door het evalueren van de resultaten van de RCT. In het algemeen liet de RCT zien dat Slimmer Zwanger een effectief programma is aangezien de deelnemers in de interventie groep een grotere verbetering van inadequaat gedrag lieten zien in vergelijking met de deelnemers in de controle groep (β=0.78, 95%CI 0.46-1.09 en β=0.83, 95%CI 0.42-1.28) voor respectievelijk vrouwen en mannen. De grootste verbeteringen werden gemaakt in voeding; vrouwen verbeterde de inname van groente en fruit, mannen verbeterde vooral de inname van fruit. Voor wat betreft de verbeteringen in leefstijl stopten vrouwen meer met roken en minderden mannen hun alcohol inname. Analyse van de voeding en leefstijl gewoontes 12 weken nadat het coaching programma was geëindigd liet zien dat de verbeteringen nog steeds aanwezig waren, wat indiceert dat de aanpassingen in gewoontes niet alleen voortduurden tijdens het programma maar dat men het ook daarna vol bleef houden. In de toekomst zal moeten worden onderzocht of deze verbeteringen in voeding en leefstijl gewoontes ook invloed hebben op de zwangerschapskans en -uitkomsten.

Bij interventie studies, is kosteneffectiviteit, naast betrouwbaarheid, kwaliteit en effectiviteit, een belangrijke determinant van de succes van een interventie. Daarom hebben we in ***Hoofdstuk 8*** middels gemodelleerde scenario's, de kosteneffectiviteit van het mHealth coaching programma Slimmer Zwanger onderzocht. Deze studie laat zien dat Slimmer Zwanger potentieel kostenbesparend is vanuit zowel een

maatschappelijk als vanuit een gezondheidszorg perspectief, aangezien het zorgt voor meer zwangerschappen tegen lagere kosten in vergelijking met reguliere zorg (ICER -€3,05 (95%CI -3,96;-539) per additionele zwangerschap).

Samenvattend is in dit proefschrift het belang van gezonde voeding en leefstijl tijdens de preconceptie periode onderbouwd. We hebben aangetoond dat de prevalentie van inadequate voeding en leefstijl gewoontes nog steeds erg hoog is onder koppels die zwanger proberen te raken, en we hebben laten zien dat Slimmer Zwanger een effectief hulpmiddel is om deze inadequate gewoontes te verbeteren. Het belangrijkste doel van dit proefschrift was het vergroten van het bewustzijn van het belang van de periconceptie periode en het verlagen van de drempel tot preconceptiezorg zodat dit meer aan bod zal komen in de gezondheidszorg. Daarnaast is het van belang om de focus meer richting de man te schuiven, aangezien we in dit proefschrift hebben aangetoond dat voeding en leefstijlgewoontes ook semenkwaliteit en vervolgens embryogenese en placentatie kunnen beïnvloeden. Tot slot, het verbeteren van voeding en leefstijlgewoontes preconceptioneel is een investering die uiteindelijk bij zal dragen aan de gezondheid van huidige en toekomstige generaties.

Addendum

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Addendum

Authors & Affiliations

F.J.M. Broekmans Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, University Medical Center, Utrecht, the Netherlands

E.M. Brouwer-Brolsma Division of Human Nutrition, Wageningen University, Wageningen, the Netherlands

M.R. van Dijk Department of Obstetrics and Gynaecology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

G.R. Dohle Department of Urology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

A.J. Eggink Department of Obstetrics and Gynaecology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

M. Goddijn Center for Reproductive Medicine, Department of Obstetrics and Gynaecology, University Medical Center, University of Amsterdam, Amsterdam, the Netherlands

B. Grace Research Department of Reproductive Health, EGA Institute for Women's Health, Faculty of Population Health Sciences,University College Londen (UCL), London, United Kingdom

J.H. Hall Research Department of Reproductive Health, EGA Institute for Women's Health, Faculty of Population Health Sciences, University College Londen (UCL), London, United Kingdom

A.C. Ham Department of Obstetrics and Gynaecology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

A. Hoek University of Groningen, Department of Obstetrics and Gynaecology, University Medical Center, Groningen, the Netherlands

N.A. Huijgen Department of Obstetrics and Gynaecology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

E. Jauniaux Research Department of Reproductive Health, EGA Institute for Women's Health, Faculty of Population Health Sciences, University College Londen (UCL), London, United Kingdom

N.F. Klijn Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, University Medical Center, Leiden, the Netherlands

R. Koedooder Department of Obstetrics and Gynaecology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

M.P.H. Koster Department of Obstetrics and Gynaecology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

J.S.E. Laven Department of Obstetrics and Gynaecology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

H.F. Lingsma Department of Public Health, Erasmus MC University Medical Center, Rotterdam, the Netherlands

R.H. Ophuis Department of Public Health, Erasmus MC University Medical Center, Rotterdam, the Netherlands

S. Polinder Department of Public Health, Erasmus MC University Medical Center, Rotterdam, the Netherlands

E.J.P. van Santbrink Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Reinier de Graaf Gasthuis, Delft, the Netherlands

E.A.P. Steegers Department of Obstetrics and Gynaecology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

R.P.M. Steegers-Theunissen Department of Obstetrics and Gynaecology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

B.H.C. Stricker Department of Epidemiology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

I. de Vos Division of Human Nutrition, Wageningen University, Wageningen, the Netherlands

S.P. Willemsen Department of Biostatistics, Erasmus MC University Medical Center, Rotterdam, the Netherlands
Department of Obstetrics and Gynaecology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

Addendum

Bibliography

Manuscripts related to this thesis

The impact of maternal lifestyle factors on periconception outcomes: a systematic review of observational studies.

E.C. Oostingh, J. Hall, M.P.H. Koster, B. Grace, E. Jauniaux, R.P.M. Steegers-Theunissen
Reprod Biomed Online 2019 Jan;38(1):77-94.

Strong adherence to a healthy dietary pattern is associated with better semen quality, especially in men with poor semen quality.

E.C. Oostingh, R.P.M. Steegers-Theunissen, J.H.M. de Vries, J.S.E. Laven, M.P.H. Koster
Fertil Steril 2017 Apr;107(4):916-923

No independent associations between preconception paternal dietary patterns and embryonic growth: the Predict study.

E.C. Oostingh, I. de Vos, A. Ham, E.M. Brouwer-Brolsma, S.P. Willemsen, A.J. Eggink, E.A.P. Steegers, R.P.M. Steegers-Theunissen
Clin Nutr. 2019 Oct;38(5):2333-2341

Potential benefits of the use of sympathomimetics for asthmatic disease, on semen quality in men of subfertile couples.

E.C. Oostingh, N.A. Huijgen, R. Koedooder, G. Dohle, B. Stricker, R.P.M. Steegers-Theunissen
Reprod Biomed Online 2020 Mar;40(3):423-428

The use of the mHealth program Smarter Pregnancy in preconception care: rationale, study design and data collection of a randomized controlled trial.

M.R. van Dijk, ***E.C. Oostingh***, M.P.H. Koster, S.P. Willemsen, J.S.E. Laven, R.P.M. Steegers-Theunissen
BMC Pregnancy and Childbirth 2017 Jan 26;17(1):46

First effective mHealth nutrition and lifestyle coaching program for subfertile couples undergoing in vitro fertilization treatment: a single-blinded multicenter randomized controlled trial.

E.C. Oostingh, M.P.H. Koster, M.R. van Dijk, S.P. Willemsen, J.S.E. Laven, R.P.M. Steegers-Theunissen

Fertil Steril 2020 Jul 30:S0015-0282(20)30418-0

Mobile health coaching on nutrition and lifestyle behaviors for subfertile couples using the Smarter Pregnancy program: model-based cost-effectiveness analysis.

E.C. Oostingh, R.H. Ophuis, M.P.H. Koster, S. Polinder, H.F. Lingsma, E.A.P. Steegers, J.S.E. Laven, R.P.M. Steegers-Theunissen

JMIR mHealth uHealth 2019 Oct 23;7(10):e13935

A mobile app lifestyle intervention to improve healthy nutrion in women before and during early pregnancy: a single-center randomized controlled trial.

M.R. van Dijk, M.P.H. Koster, ***E.C. Oostingh***, S.P. Willemsen, E.A.P. Steegers, R.P.M. Steegers-Theunissen.

J Med Internet Res. 2020 May 15;22(5):e15773

Other manuscripts

Outcome of ovulation induction with Clomiphene citrate in women with PCOS; patient characteristics and the influence of ethnicity.

C. Meun, ***E.C. Oostingh***, J.S.E. Laven, Y.L. Louwers

Submitted at BJOG.

Addendum

PhD portfolio

Name PhD student	Elsje Cornelia Oostingh
Departments	Obstetrics and Gynaecology
Research School	NIHES
PhD Period	2014-2018
Promotors	Prof. dr. R.P.M. Steegers-Theunissen
	Prof. dr. J.S.E. Laven
Co-promotor	Dr. M.P.H. Koster

PHD TRAINING

General courses

Biostatistical Methods I: Basic principles (Nihes)	2015, September
Basic principles of R	2016, January
e-BROK course (NFU BROK Academy)	2016

Participation and collaboration

Rotterdam periconceptie cohort (PREDICT)	2014 - 2018
Slimmer Zwanger Rotterdam trial – RCT	2014 - 2016
Slimmer Zwanger Nederland trial (IVF) – RCT	2014 - 2018
Regiocoördinator Perined Zuid-West NL	2014 - 2017

Study site presentations

AMC Amsterdam, Slimmer Zwanger	2015, January
UMC Utrecht, Slimmer Zwanger	2015, January
Antonius Nieuwegein, Slimmer Zwanger	2015, April
UMC Groningen, Slimmer Zwanger	2015, June
Reinier de Graaf Gasthuis, Slimmer Zwanger	2015, July
MCL Leeuwarden, Slimmer Zwanger	2015, July
UMC Utrecht, Slimmer Zwanger	2015, November
LUMC Leiden, Slimmer Zwanger	2016, March

Attended seminars, conferences and workshops

Weekly research meeting of the department of obstetrics and gynaecology	2014-2018
Three-monthly research meetings Rotterdamse Gynaecologen Opleiding Cluster (RGOC)	2015-2019
Annual Wladimiroff award Meeting, department of obstetrics and gynaecology	2015-2019
Annual Sophia Research Day	2015-2018
Rhenen, landelijke inspiratiedag Perined	2015, June
Rotterdam, Science Congress	2015, September
Utrecht, jaarcongres Perined	2015, October
Cape Town, 9th DOHaD World Congress	2015, November
Apeldoorn, jaarcongres Perined	2017, October

Presentations at (inter)national conferences

Den Haag, ZonMW Kennispoort congres	2014, November
Orlando, The 64th Annual Meeting of the Society for Reproductive Investigation (SRI)	2017, March
Rotterdam, Regionale audit Perined	2017, June
Rotterdam, 10th DOHaD World Congress	2017, October
Amersfoort, NVOG 52e Gynaecongres	2017, November
Rotterdam, Refereeravond neonatology, Sophia	2017, November
Utrecht, ZonMW Kennispoort congres	2018, January
Heerenveen, Symposium digipoli Tjongerschans ziekenhuis	2018, February
San Diego, The 65th Annual Meeting of the Society for Reproductive Investigation (SRI)	2018, March
Wladimiroff award meeting (Award winning lecture)	2018, April
Rotterdam, 22e Nederlands-Vlaams Doelencongres	2019, April

Organisation

CJG refereeravond	2014, November
Regionale audit regio Zuid-West Nederland	2015, February
Regionale audit regio Zuid-West Nederland	2017, June

Media

NTOG, artikel, "Update Slimmer Zwanger-studie"	2015, May
RTV Rijnmond, interview Slimmer Zwanger	2015
NTOG, artikel "Slimmer Zwanger: eerste effectieve mHealth-coachingprogramma"	2020, April

Supervised (medical) students

Anne Edixhoven, Erasmus MC 2015

Associations between paternal factors, including semen parameters, and first trimester CRL, using three-dimensional ultrasound scans.

Iris de Vos, Wageningen University 2017

Associations between preconception paternal dietary patterns and first trimester embryonic growth using longitudinal embryonic volume and crown-rump length measurements.

Lecturing

Teaching students VAR	2015-2017
Coaching bachelor students, KBP	2016-2018
Lecture in the minor 'mystery of creations'	2017, October

Perinatale Audit Nederland (PAN) / Perined

Audits in 2014 2

Audits in 2015 32

Audits in 2016 26

Audits in 2017 30

Audits in 2018 3

Addendum

About the author

Elsje Cornelia (Eline) Oostingh was born on the 23rd of November 1985 in Katwijk as the first child of Henk and Hubertha Oostingh. Her birth took place at home and didn't go completely well, so Eline was admitted to the hospital immediately after her birth and had to stay there for two weeks. Whether this was the reason for her love for the profession of doctor remains difficult to say, but for us as parents it is clear that she has developed a great fondness for the profession of doctor since childhood.

Eline went through primary and secondary school smoothly and obtained her VWO diploma in 2005 at the Pieter Groen college in Katwijk. As she was determined to become a doctor, Eline did not want to be dependent of being drawn in order to start the study, so she successfully took part in the decentralized selection at the Erasmus University Medical Centre Rotterdam and started her medical training in 2005.

During medical school Eline worked at the Trombosedienst in Leiden, where she visited many patients with her bright red service car, and at the student team of the obstetrical ward of the Sophia Children's hospital. She performed her medical research internship abroad for 1 year at the Elisabeth hospital in Willemstad, Curaçao where she studied young patients with a Fontan circulation because of hypoplastic left heart syndrome.

Over the years her enthusiasm for obstetrics and gynaecology expanded, therefore she completed the last part of her internships at the Gynaecology department of the Albert Schweitzer hospital in Dordrecht and continued there as a resident (ANIOS) and one year later at the Erasmus MC. In 2014 she started her PhD-training on the Smarter Pregnancy IVF-trial. She conducted the study under the guidance of Prof. dr. R.P.M Steegers-Theunissen, Prof. dr. J.S.E. Laven and Dr. M.P.H. Koster which resulted in this PhD thesis.

In September 2016 she married Jules van Heeswijk and they became proud parents of two little boys in August 2018. Since January 2019 Eline started her obstetrics and gynaecology residency training.

As parents, we have great respect for Eline's perseverance and relentless energy to learn to understand and practice her profession to perfection. Her drive and passion to provide optimal support to patients and, in particular, the social aspect that she puts in practicing her profession, makes us proud of her as a person.

We wish her all the success in her further career as a social and compassionate doctor.

Henk en Hubertha Oostingh

Addendum

Acknowledgements

Dankwoord

‘Laat me je
de stad tonen
waarvan ik ben
gaan houden’

Promoveren is meer dan artikelen schrijven en ze publiceren. Het vergt doorzettingsvermogen, een grote mate van flexibiliteit en om kunnen gaan met teleurstellende wetenschappelijke uitkomsten. Maar voor mij is het bovenal een bijzonder en machtig mooie periode geweest waarin veel nieuwe vriendschappen zijn ontstaan, gave reizen zijn gemaakt, de nodige liters koffie zijn gedronken en cakevandeweek's zijn gegeten. En dit alles in de mooie stad die ik heb leren waarderen en waar ik me thuis ben gaan voelen.
Buiten dit alles was dit proefschrift niet tot stand gekomen zonder de deelname van alle patiënten aan de Slimmer Zwanger IVF-trial, waarvoor heel veel dank. Een aantal mensen wil ik in het bijzonder bedanken.

Allereerst mijn (co)promotoren. Mijn eerste promotor, **professor dr. Steegers-Theunissen, beste Régine**, ik ben je dankbaar dat je wetenschappelijke potentie in mij zag en me een plek aanbood binnen jouw team. Eerlijk gezegd, weet ik achteraf niet of ik toen wel helemaal wist waar ik aan begon. Maar hoe dan ook, het is een fantastische tijd geweest waarin ik enorm veel heb geleerd. Dank dat je mij het vertrouwen gaf om de Slimmer Zwanger IVF-trial verder op te zetten en te leiden. Ik vind het inspirerend hoe je altijd nieuwsgierig blijft naar nieuwe wetenschappelijke inzichten en je hier met hart en ziel voor inzet.

Mijn tweede promotor, **professor dr. Laven, beste Joop**, na mijn ietwat desastreus verlopen wetenschappelijke stage tijdens de studie, gaf je me als ANIOS in het Erasmus MC met de woorden "Ik geef niet snel iemand een tweede kans, dus laat maar zien wat je waard bent", de mogelijkheid om je op wetenschappelijk vlak opnieuw voor me te winnen. Ik hoop dat het is gelukt.
Hoe dan ook stemde je een jaar later, bij de start van mijn promotieonderzoek, in om m'n tweede promotor te zijn, en daar ben ik je dankbaar voor. Bij jou mocht ik alles zeggen, je gaf ruimte voor discussie als ik het ergens niet mee eens was, ook al veranderde dit de uitkomst meestal niet. Dank voor je vertrouwen in m'n eigen kunnen en je sturing wanneer dit nodig was.

Geachte co-promotor, **dr Koster, beste Wendy**, of zal ik maar gewoon zeggen; **lieve baas**. Na een klein jaar op m'n eigen wetenschappelijke kunsten te zijn aangewezen was jij daar, en ging het me plots voor de wind. Ik genoot van de discussies die we konden voeren over de statistiek of inhoudelijk over een paper en ook al kreeg ik m'n zoveelste versie volledig roodgekleurd door al jouw aanpassingen terug, toch gaf je me altijd het gevoel dat ik het heel goed had gedaan. Zo fijn!
Naarmate we elkaar beter leerden kennen bleek dat we niet alleen op de 22e goed met elkaar overweg konden maar dat dit ook meer dan prima ging buiten werktijd. Onze professionaliteit kwam daarbij nooit in het geding, we switchen moeiteloos van gezellig samen bier drinken naar een strenge baas als ik m'n deadline weer eens niet dreigde te halen. Ik ben je dan ook intens dankbaar voor al je hulp die je me gegeven hebt tijdens mijn promotie en dat je m'n klankbord was. Ik hoop dat we elkaar nooit uit het oog zullen verliezen.

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